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"HYDROPHOBICITY IN POLYSACCHARIDE GELATION"

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"From a certain point of view the universe seems

to be composed of paradoxes. But everything resolves.

That is the function of contradiction."

Ben Okri, 1991.

Acknowledgements

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Abstract

The role of hydrophobic substituents on the gelation mechanism of highly esterified pectin and the cellulose derivatives methylcellulose and hydroxypropylmethylcellulose (HPMC) has been explored by monitoring the behaviour of the amphiphilic polysaccharides in varying combinations of an ethylene glycol/water solvent.

The gelling ability (mechanical spectroscopy, visual observation) of very highly esterified (~ 100%) pectin in high concentrations of ethylene glycol (>60%) is greatly reduced, however, the polymer still undergoes conformational ordering (CD, OR). A model for gel formation involving a two stage process has been proposed, comprising adoption of the ordered structure stabilised by hydrogen bonding between OH groups of contiguous polysaccharide chains, followed by (or coincident with) aggregation of the ordered sequences by 'hydrophobic' clustering of the fundamental structural subunits to form the three dimensional gel network. It has been found that ethylene glycol promotes the first stage (ordering) but is antagonistic to the second (aggregation).

The reversibility (mechanical spectroscopy) of the thermo-gelling cellulose derivatives can be largely abolished in the presence of ethylene glycol (40% for methylcellulose, 10% for HPMC), attributed to solubilisation of the proposed ordered 'bundle' structure at low temperatures removing the enthalpic advantage (DSC) of gel melting. The increased sensitivity of HPMC to modification of the solvent environment is due to the presence of the polar hydroxypropyl substituent causing an inceptive destabilisation of the 'bundle' structure. It is suggested that gelation is driven by the entropic advantage of melting-out 'cages' of structured water surrounding the hydrophobic groups giving rise to intermolecular 'hydrophobic' aggregation.

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List of symbols

$[\eta]$	intrinsic viscosity
η_{rel}	relative viscosity
η_{sp}	specific viscosity
η^*	dynamic viscosity
G'	elastic modulus
G"	viscous modulus
G*	complex modulus
CD	circular dichroism
$[\theta]$	molar ellipticity
OR	optical rotation
$[\phi]$	molar rotation
Tm	transition midpoint temperature
σ	shear stress
γ	shear rate
ω	oscillatory frequency
δ	phase lag
HM	high methoxy
VHM	very high methoxy
DE	degree of esterification
ΔG	free energy change
ΔH	enthalpy change
ΔS	entropy change
MS	molar substitution
DS	degree of substitution
HPMC	hydroxypropylmethylcellulose
IPT	incipient precipitation point
CP	cloud point
IGT	incipient gelation point

Chapter 1: INTRODUCTION

1.1 General Introduction

The aim of this project is to explore the role of hydrophobic substituents on the gelation of highly esterified pectin and the cellulose derivatives methylcellulose and hydroxypropylmethylcellulose. Both polymer types contain hydrophobic groups within their molecular structure, and these may play a major role in chain association by hydrophobic bonding.

The nature of the hydrophobic effect derives from the very strong attractive forces between water molecules in an isotropic arrangement. The addition of any solute or ion will obviously distort or disrupt the arrangement. Polar molecules are capable of forming strong bonds with water molecules (dipolar attraction and hydrogen bonding) whereas non-polar hydrophobic groups do not form strong associations with water molecules.

When amphiphilic molecules, containing both polar and non-polar constituents, are dissolved in water they achieve segregation of their hydrophobic portions from the solvent by self-association. This study investigates the consequence of such amphiphilic behaviour in gelling polysaccharides.

Much work has been done on characterising high methoxy pectins and the factors influencing their gelation. Proposals have been made as to the nature of their ordered association in the solid state, from X-ray diffraction studies of dried fibres. However, the structure of the gelled network and the role of solvent within that structure is still little understood.

The cellulose derivatives have more recently gained interest as commercial additives and although much is known about the chemistry of their production, little is known about the process of gelation. These polymers gel on heating, unlike the high methoxy pectins which develop network structure on cooling.

The research reported in this thesis, however, has revealed some underlying generalities of behaviour, particularly in response to changes in solvent, which are likely to have wider applicability in explaining and predicting the properties of amphiphilic polymers.

1.2 Introduction to Pectin

1.2.1 Origin

Pectin is the collective name for a group of polysaccharides extracted from edible plant material. Found in the middle lamella of plant tissues and also in the primary cell wall, it is a structural carbohydrate and is to a large extent responsible for firmness and form-retention of the tissues as well as softening during ripening. Industrial pectin is extracted from citrus peel or apple pomace under mildly acidic conditions, isolated by precipitation and ground to a fine powder of standardised properties. Due to its presence in fruit and vegetables it also forms part of the diet of man and attracts great attention as dietary fibre either in the native extracted form or in a purified form as a constituent of dietary fibre preparations.

The name is derived from the Greek word 'Πεχτος 'meaning "to congeal" or "solidify" and it is this property that has given pectin its world-wide importance as a gelling agent and stabiliser for the food industry. The discovery of the chemical compound was made by Vauquelin in 1790 but Braconnot (1825) was the first to characterise it as the component of fruit responsible for gel formation. For centuries it has been used in preserving fruit by boiling down with sugar to form a jelly. Unlike most other food hydrocolloids, pectin shows optimum heat stability under conditions of high acidity and is therefore a perfect candidate whenever a texturizer or stabiliser is required in an acidic food product (Christensen, 1986). It is primarily used in the production of jams and jellies; other applications include bakery fillings and glazes, fruit preparations, stabilising oil-in-water emulsions in sauces, and in dairy products where it acts as a protective colloid preventing the coagulation of casein.

1.2.2 Structure and Conformation

A complete understanding of structure function relationships in pectin has not yet been achieved. This may reflect the degree of heterogeneity of the pectins depending upon their origin, extraction and subsequent treatment.

The pectin molecule is found to exist in nature as a branched, partially methyl-esterified chain where the backbone is based on a repeat sequence of 1,4-linked α-D-galacturonate residues. The physical properties and biological function of polysaccharides are more dependent on the nature of the linkages and the arrangement of the bonds to and from each residue than on the chemical identity of the individual component residues. Since the geometry of the individual sugar rings is essentially fixed, the chain conformation of a homopolysaccharide such as pectin is defined by two variables only, which are the angles of rotation about the bonds to each glycosidic oxygen, ϕ and ψ (Rees, 1977).

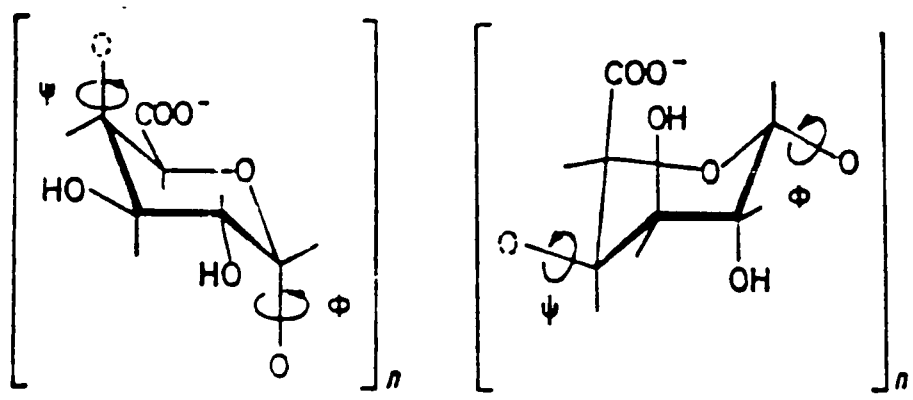


Figure 1.1 Residue conformation in polysaccharide chains (From Rees, 1977).

These angles may be fixed and have the same values at all positions along the polymer chain to give ordered chain conformations, as found in the solid state, or be constantly fluctuating to give the "random coil" behaviour typical of polymer solutions.

The geometric arrangement of the chain is determined by the character of the covalent linkages between each residue. The basic structure of each residue is a six membered pyranose ring in a 'chair' conformation. The hydroxyl groups and carbon 6 may be attached to the ring carbons either in equatorial positions around the periphery of the ring or in axial positions lying vertically above or below the ring. Steric hindrance prevents certain conformations and encourages others. In stable chair forms the bulky C(6) group always lies away from the ring equatorially to prevent crowding by other substituents.

In α-D-galacturonate the OH groups at carbons 1 and 4 are in the axial position. Linkage into pectin chains therefore involves diaxial bonds diagonally across the sugar ring, with the bonds to and from each residue being separated by the full width of the ring. This gives rise to a highly buckled ordered chain conformation which, when packed together, forms large interstices between chains.

The chain is interrupted periodically by the insertion of 1,2-linked rhamnose residues. These do not fit into the regular linear conformation and cause the chain to kink (Rees and Wight, 1971). This interruption of the ordered sequence is an important factor in the gelation process.

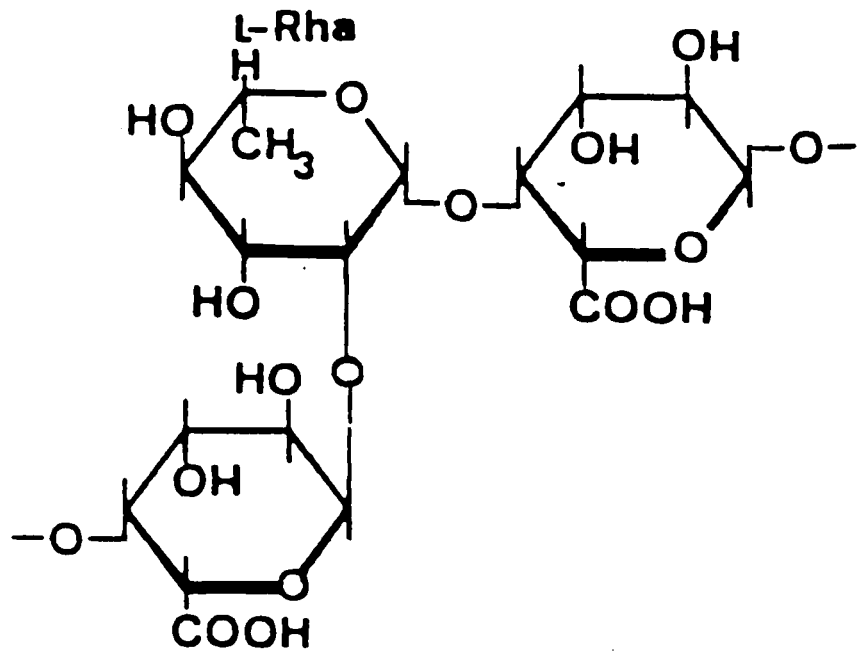


Figure 1.2 Kink produced by 1,2-linked α-L rhamnose (From Pilnik, 1990).

L-Arabinose and D-galactose are covalently bound to the rhamnose residues of the polymer backbone as complicated neutral sugar side chains linked through equatorial α-1,4-glycosidic bonds. In carefully extracted pectins from many fruits and vegetables one finds 1 to 4 molecules of rhamnose and 10 to 15 molecules of arabinose and galactose per 100 molecules of galacturonate (Pilnik, 1990). However, the industrial preparation of pectin extracts causes a significant fall in the number of neutral sugar side chains due to acid hydrolysis. The treatment does not affect the 1,2-linked rhamnose residues inserted within the polygalacturonan backbone, but the bond between C(1) in rhamnose and C(4) in galacturonic acid is less stable than the other glycosidic linkages and is the point of cleavage under conditions of partial acid hydrolysis.

The molecular weight of pectin varies in accordance with the nature of the raw material, the extraction procedure and the treatments employed in its isolation. The average MW of commercially available pectin is in the range 50,000 to 150,000 Daltons (Owen *et al.*, 1946).

1.2.3 Classification of Pectins

There are two main types of pectin; high methoxy pectin and low methoxy pectin, which occur naturally or can be produced industrially. They are classified according to the degree of methyl esterification occurring at the carboxylate group on C(6).

The degree of esterification (DE) is defined as the ratio of esterified galacturonic acid units to total galacturonic acid units in the molecule (Christensen, 1986). Pectins with a DE above 55% are high methoxy pectins (HM Pectins), and those with a lower DE are low methoxy pectins (LM Pectins). In un-modified pectin as it is extracted from plant tissue the DE is in the range of 10%, that found in strawberries, to 60%, which is the level commonly found in apple pulp and citrus peel (Coultate, 1989).

Non-esterified residues may exist in the undissociated form (COOH), or in the dissociated form (COO^-) with ammonium, potassium, sodium or other counterions depending upon the source. The pKa of pectin is about 3.6; under most biologically and technologically relevant conditions the pH exceeds 3.6 and pectin is found as a predominantly charged polymer with individual chains repelling each other.

High methoxy pectins require a minimum content of soluble solids (above 55%) and an acid pH, around 3.0, in order to form gels where the binding forces between the chains are hydrogen bridges and hydrophobic forces between methoxyl groups. The low water activity reduces the amount of bound water which would otherwise disrupt the fragile affinities of the junction zones (see 1.2.5) and the acidic pH ensures that few of the galacturonate residues are present in the ionised COO^- form, so reducing electrostatic repulsion and allowing association of the chains. The rate of gelation of high methoxy pectins is dependent on the degree of esterification, with pectin having an ester content in

the range 70-75% being very rapid set, whereas slow set pectins have typical ester contents around 50-55%.

De-esterification of naturally occurring high methoxy pectin is carried out industrially by treatment at mildly acidic or alkaline conditions to form low methoxy pectins. There are four main methods of de-esterification available, summarised by Taylor (1982) as being treatment with acid, alkali, enzymes or ammonia. These low methoxy pectins find their applications in industry in non-acidic food products due to a different gelling mechanism from that of high methoxy pectin. They form stable gels only in the presence of divalent metal cations, most notably calcium. They can tolerate much greater changes in pH and can form strong gels at much lower soluble solids content than high methoxy pectins. Low methoxy pectins can also be classed as rapid and slow set depending on their calcium reactivity. Low methoxy pectins having ester contents in the range 25-35% are characterised as highly calcium reactive, rapid set, whilst those in the region 35-45% are slow set, less calcium reactive pectins. Low methoxy pectins were formerly used in relishes and salad dressings before the advent of the weak gel bacterial polysaccharides. Now they have particular application in the formation of jams and jellies for the low calorie food market. This is due to their reduced dependence upon soluble solid content for gelation and the fact that pectin remains undigested in the human stomach, only becoming hydrolysed by the gut flora in the large intestine, so conferring a low net calorific value (Christensen, 1986).

1.2.4 Formation of Low Methoxy Pectin Gels

Low ester pectins are highly charged due to the free carboxyl group present on Carbon 6. For gel formation to take place counterions must be added to preserve electrical neutrality and offset electrostatic repulsions between the charged polymer chains (Kohn and Furda, 1967). The counterion most commonly incorporated into the associating structure is the divalent cation, calcium, whose ionic radius is close to the smallest size of the cavities created between the buckled ribbon sequences in a regular 2-fold configuration. Kohn (1975) has studied the binding of divalent ions to polygalacturonate and concluded calcium binding cannot be attributed to simple electrostatic attractions, but involves intermolecular chelate binding.

The stoichiometry of interchain binding was investigated by Morris *et al.* (1978), measuring calcium binding under swamping conditions of a univalent counterion (tetramethylammonium $(CH_3)_4N^+$). The proportion of calcium resistant to displacement by the univalent ions was found to be 50% of the total stoichiometric requirement of the polygalacturonate chain sequences, which is evincive of tight dimeric binding sites formed between chains of 2_1 symmetry. Grant *et al.* (1973) have studied calcium binding using circular dichroism and have shown that the spectral changes on gelation are virtually identical in form and magnitude but opposite in sign to those observed for calcium polyguluronate which is known to exist in the 2_1 conformation. This shows the mirror image relationship of the two compounds and indicates an analogy between the formation of junction zones (i.e. regions of chain association) in each species by dimerization of chain sequences in the 2_1 configuration. The molecular appearance of the junction zones leads to the term 'egg box' binding.

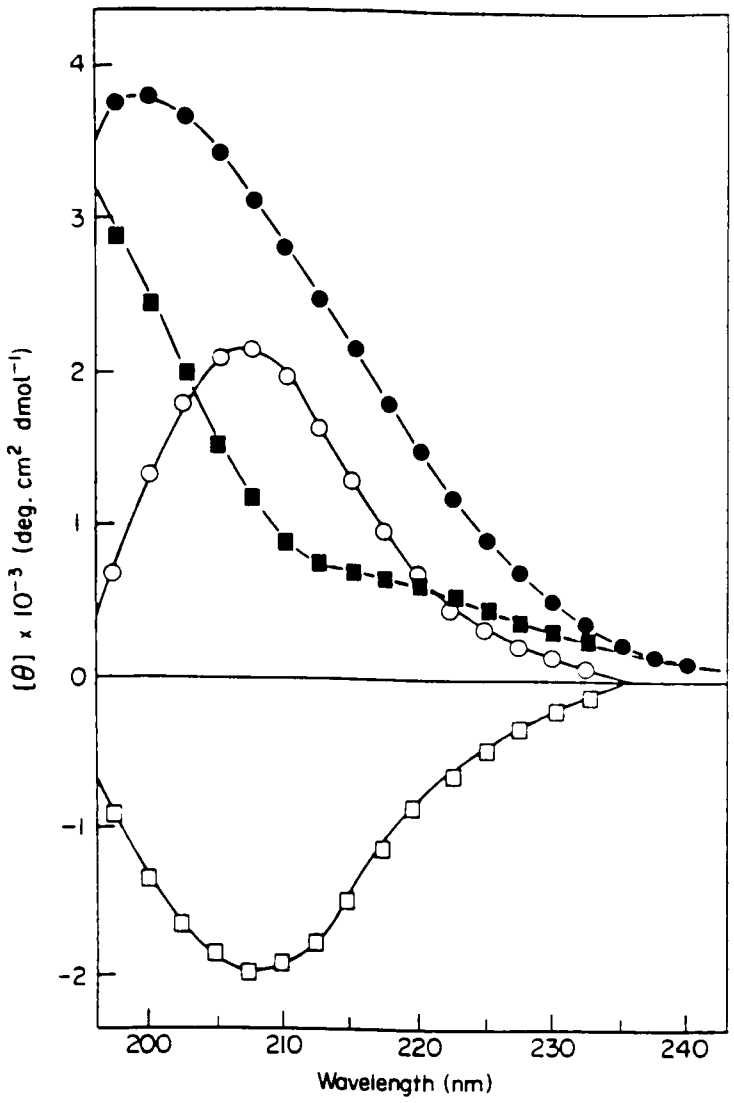


Figure 1.3 Changes in polyuronate circular dichroism behaviour with cation binding. Spectral changes (○) between Na^+ solution (●) and Ca^2 gels (■) for polygalacturonate chains are similar in form and magnitude but opposite sign to those observed for polyguluronate (❑). (From Morris *et al.*, 1982).

The length of uninterrupted galacturonan sequences capable of taking part in dimerization has been studied by Powell *et al.* (1982) to probe the primary structural requirements for junction zone formation in calcium pectate gels. Sequence length between rhamnose 'kinks' is found to be around 25 residues, with little variation between pectin samples. A model for pectin has been proposed comprising smooth regions of pure homogalacturonan interrupted by short 'hairy' regions rich in rhamnose and side chains (Pilnik, 1990).

The junction zones are formed between the smooth homogalacturonan regions and are terminated by the presence of kinking 1,2-linked α-L-rhamnosyl groups in the 'hairy' regions which are incompatible with the ordered associating sequences (Powell *et al.*,

1982) or alternatively by methyl esterification of the C(6) carbon groups. This effectively neutralises the charge on the polymer so that it can no longer bind calcium and the dimerised egg box structure cannot form. Prevention of complete dimerisation allows each chain to participate in associations with a number of different galacturonate sequences and so leads to a three dimensional gelled network held together by junction zones with solubilising regions to prevent total aggregation and precipitation.

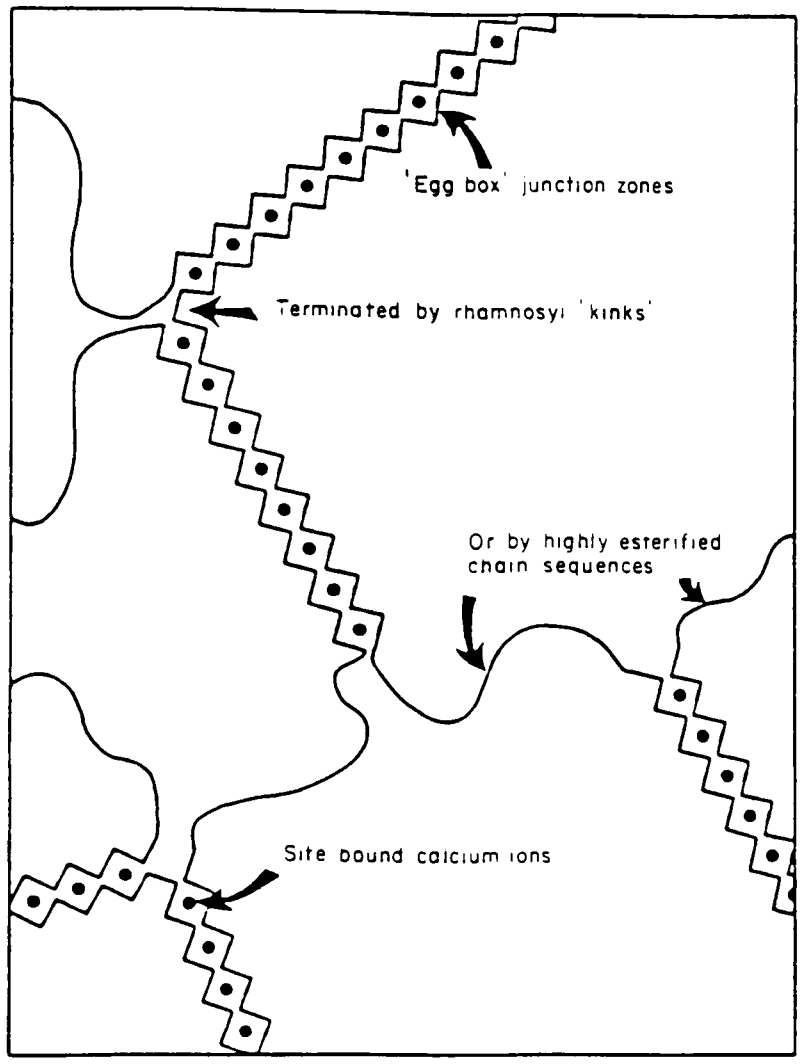


Figure 1.4 Schematic representation of calcium pectate gel (From Morris, 1986).

If calcium is present in excess of the requirements for 1:2 calcium: polymer binding, then calcium-induced aggregation of the preformed dimers may occur. This leads to brittle gels with a strong tendency to syneresis, and eventually the structure may collapse due to extensive crystallisation and precipitation of the hydrocolloid (Powell *et al.*, 1982).

1.2.5 Formation of High Methoxy Pectin Gels

If the degree of esterification becomes so great that the majority of residues are neutralised, then calcium is no longer required for chain association and the polymer gels by a different mechanism. The chains can pack together without the need for stabilising cations. Pectin extracted from the plant is never 100% esterified so there will always be a number of charged residues within each pectin sample. In order to overcome the repulsion effects of this charge the pH is lowered, the carboxyl groups become protonated and the charge is suppressed so allowing the chains to come together. Gelation also requires a reduction in the water activity brought about by the addition of co-solutes such as sucrose or ethylene glycol. Mitchell (1976) has suggested that co-solutes play an active role in stabilising the gels by the formation of secondary links by hydrogen bonding and that these contribute to the overall gel strength. As well as neutralising the chains, methyl groups are thought to actively contribute to the stability of the gel network and the greater the degree of esterification, the faster the gel will set. The limiting factor for aggregation and precipitation of the polymer is again the presence of rhamnosyl insertions incompatible with inter-chain association.

A model for the packing arrangement of high methoxy pectin has been suggested by Walkinshaw and Arnott (1981b) from X-ray diffraction studies on dried fibres of pectin where the polymer exists as a 3_1 right handed helix. Computer modelling indicates a triangular pattern of polymer chains held together by bonding to columns of water in one set of channels and hydrophobic attractions between methyl groups in a second set of channels running through the centre of the crystalline aggregate.

1.3 Introduction to Cellulose Derivatives

1.3.1 Cellulose

Cellulose is the most plentiful renewable resource in the world (Glicksman, 1986). It is a major constituent of all land plants and also found in substantial quantities in a number of sea plants. It is responsible for providing the structural framework of the tissues and is located in the plant cell walls. It takes on a very close packed arrangement which is not easily disrupted and is insoluble in water.

Cellulose is an unbranched linear molecule consisting of a backbone of glucan comprising β-D-glucopyranosyl units with 1,4-diequatorial linkages resulting in an extended ribbon geometry.

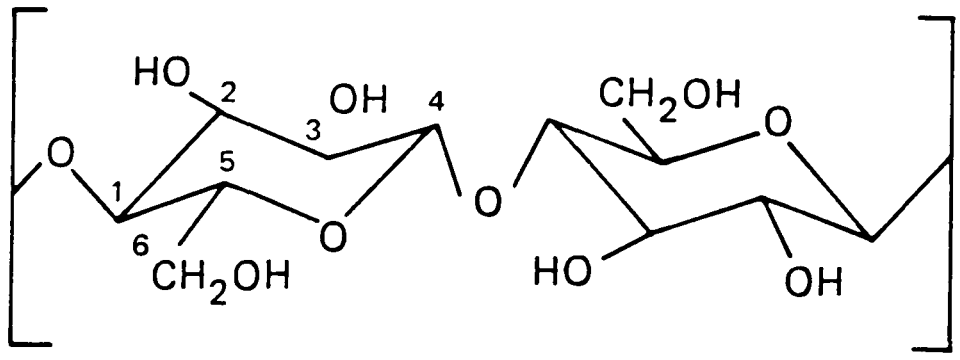


Figure 1.5 Structure of cellulose (From Grover, 1986).

Each pyranosyl ring is in the $^4C_1$ chair form and the whole polymer molecule takes on a two-fold screw axis. There are between 2000 and 3000 units in a single polymer chain. Cellulose molecules exist in a regular packing arrangement consisting of bundles of about 2000 chains termed microfibrils, which are then organised into parallel alignment of about 400 microfibrils giving rise to a conformationally robust, insoluble crystalline structure.

The stability and insolubility of cellulose is thought to be due to a co-operative array of non-covalent interactions, including lateral association by hydrogen bonding between chains. Two analogous compounds, oat glucan and seed amyloid show water solubility due to the presence of a 1,3-linkage every fourth unit in the former and the occasional addition of a single branch unit linked 1,6 to the main chain in the latter. Both of these modifications are absent in cellulose.

Cellulose is completely indigestible by the enzymic secretions of animals, including man, due to the absence of the cellulase enzyme. However, gut flora present in the lower bowel of man and in the rumen of the ruminant animals are able to break down and ferment cellulose. This is an important addition to the nutrition of the ruminants, but the energy remains largely un-utilised by man.

Due to the relative inertness and insolubility of cellulose its application and usefulness is somewhat limited. In order to solubilise cellulose it must undergo chemical processing and modification which gives rise to a number of important derivatives with great scientific interest and industrial value. The use of these derivatives in foods is due to their ability in imparting properties such as adhesion, emulsification, viscosity control, gel formation and moisture retention.

1.3.2 Chemical Derivatisation

There are many different derivatives of cellulose that can be formed depending upon the chemical process used. This study considers only two of the variants, namely, methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC).

These ethers of cellulose are produced by the substitution of methyl or hydroxypropyl groups in place of the hydroxyl groups found on each glucose residue. There are three hydroxyl groups per residue available for substitution as can be seen in Figure 1.6.

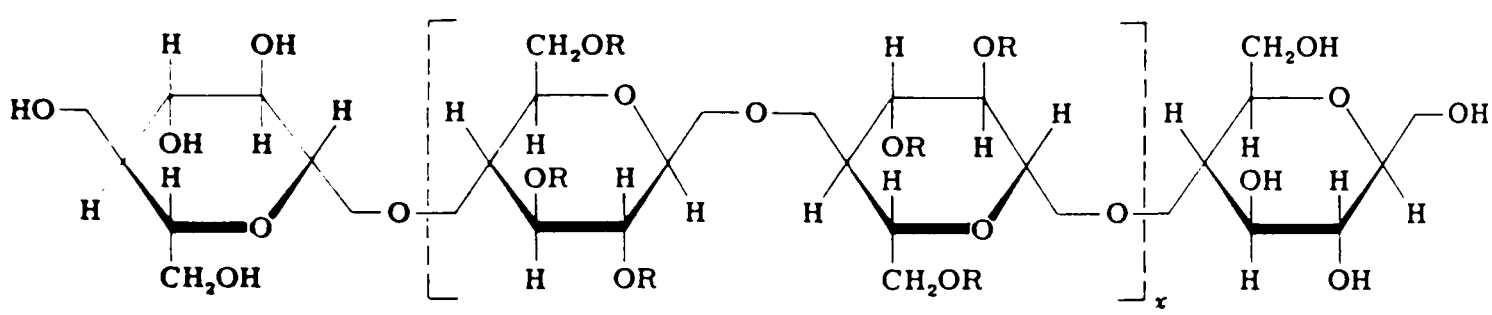
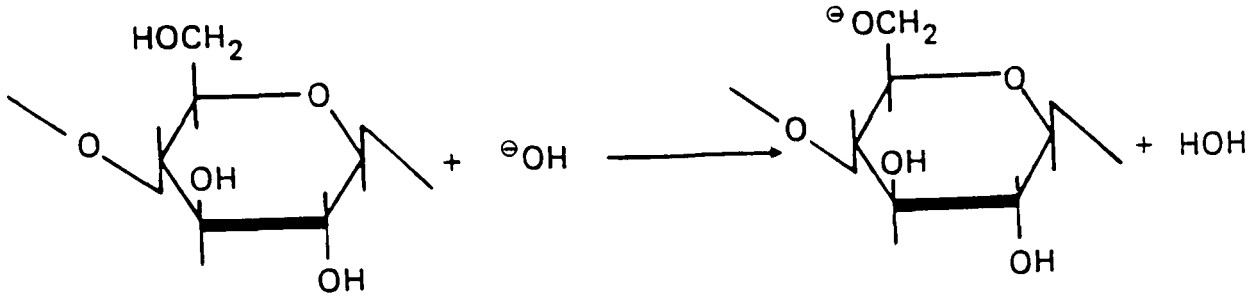
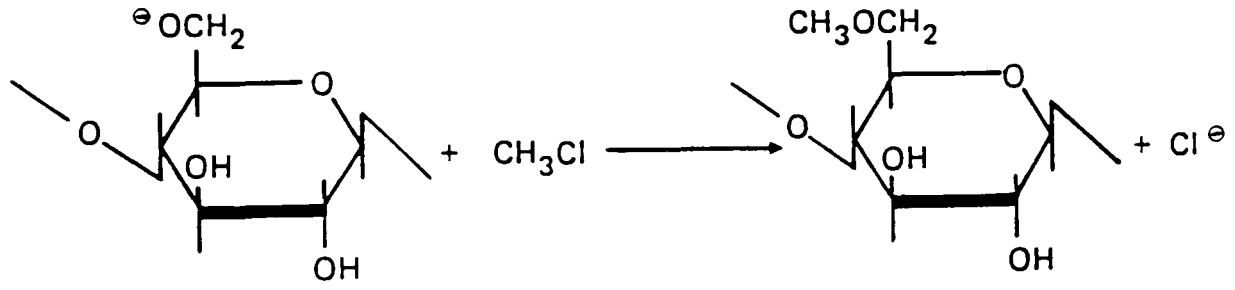


Figure 1.6 Structure of cellulose derivatives (From Glicksman, 1969), where R represents the substituents CH_3 in methylcellulose and CH_3 or CH_3-HOCH-CH_2 in hydroxypropylmethylcellulose.

The degree of substitution, DS, is defined as the average number of hydroxyl groups per anhydroglucose unit that have been substituted, and has a maximum value of three. The presence of hydroxypropyl substituents provides a further hydroxyl group which can again be substituted forming short side chains. The level of hydroxypropyl substitution is defined by the number of moles of substituent per mole of glucose and is termed the molar substitution, MS.

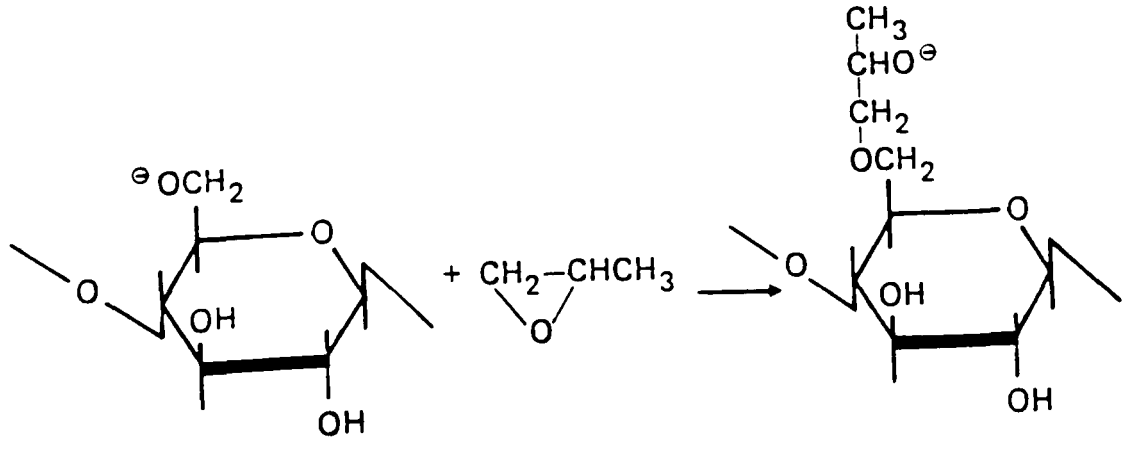
The first stage in production of the alkyl and hydroxy alkyl cellulose derivatives is the formation of alkali cellulose by treatment of powder, chips, shreds or sheets of cellulose with sodium hydroxide to swell and distend the fibres.





For the formation of MC the alkali cellulose is reacted with methyl chloride, CH_3Cl.

In the production of HPMC there is a further reaction with propyl oxide.



Varying the ratios and amounts of methoxyl and hydroxypropyl units gives a vast array of possible products each with different chemical characteristics. Table 1.1 lists those available from the Dow Chemical Company, according to DS and MS and molecular weight as determined by viscosity. The viscosity of MC and HPMC is measured for standard 2% solutions at 20°C; standard commercial products range between 10cps (13,000 MW) to 19,000 cps (140,000 MW).

1.3.3 Solubility

As previously mentioned, cellulose is insoluble due to its tight crystal packing and hydrogen bonding. Etherification of the polymer disrupts the crystallinity and forces the cellulose chains apart by the presence of the bulky substituent groups, leaving the unsubstituted hydroxyl groups, normally tied up by intermolecular hydrogen bonding, available for hydration (Greminger, 1973). Also, because of the loss of hydroxyl groups due to substitution, the degree of internal association by hydrogen bonding is greatly reduced and the whole molecule becomes soluble. The nature of the effective solvent changes with the degree of substitution; low DS polymers are soluble in aqueous alkali solutions, water solubility occurs in polymers around DS 1.4 and highly substituted DS 2.6 ethers are soluble in polar organic solvents (Grover, 1986).

Within the broad ranges indicated above, however, polymers with the same overall DS can show substantial differences in solubility (and thermogelation behaviour described in the following section) depending on the precise conditions used for their manufacture. In particular, Savage (1957) has suggested, from measurements of the solution viscosity of various cellulose ethers, that solubility in water is dependant on a uniform distribution along the polymer chain of unsubstituted or sparingly substituted regions that are capable of interacting with water by hydrogen bonding.

1.3.4 Thermogelation

MC and HPMC undergo gelation at high temperatures and revert to solutions on cooling. The temperature course of structure formation and dissociation for methylcellulose was first mapped out by Heymann (1935), from measurements of solution viscosity in a simple Ostwald viscometer. More recently, the same approach was adopted in a comprehensive investigation by Sarkar (1979), but using rotational viscosity measurements. The viscosity profile obtained for MC is shown in Figure 1.7.

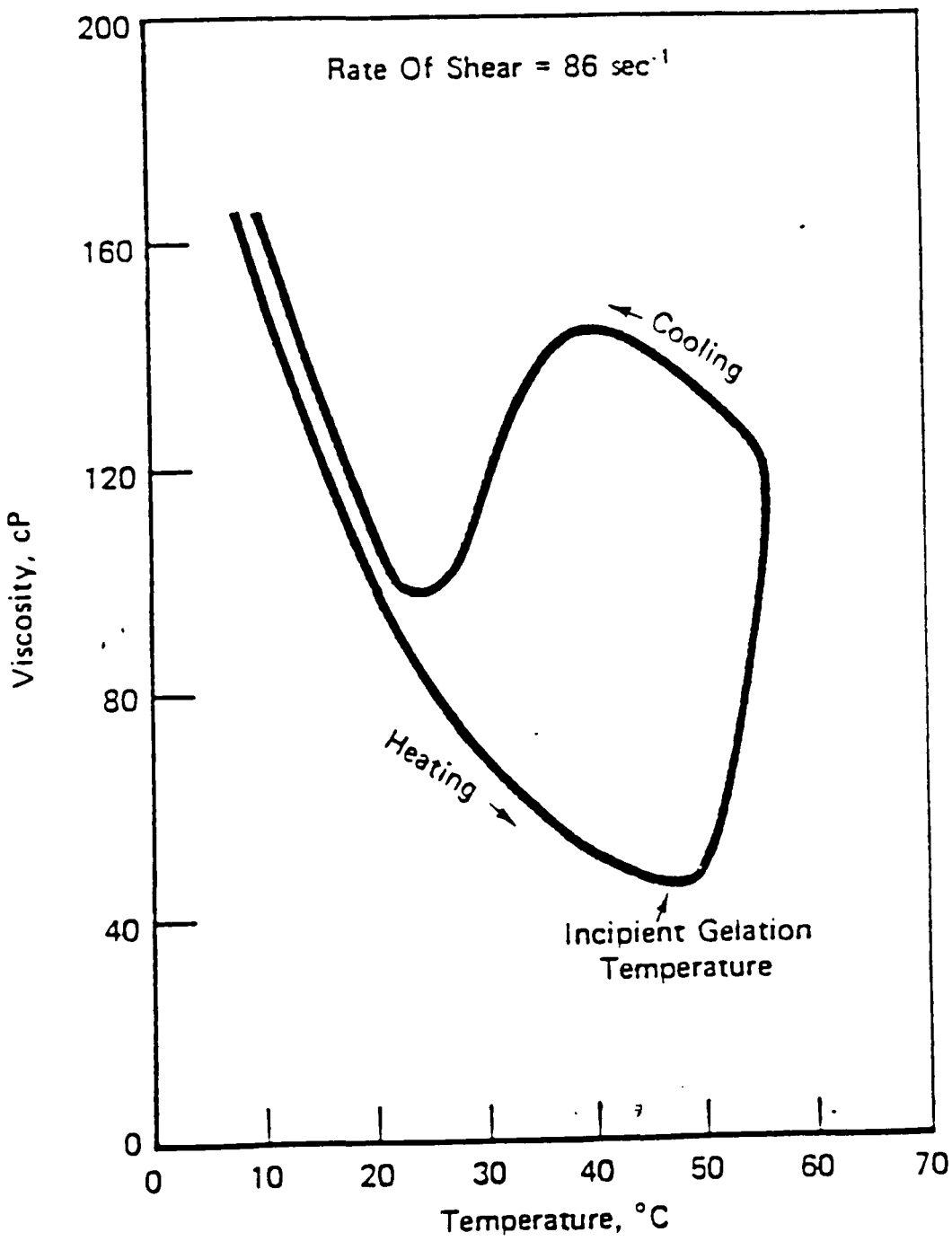


Figure 1.7 Gelation of 2% aqueous solution of methocel A100 on heating at 0.25°C/min (From Sarkar, 1979).

The gels are completely reversible but show a marked thermal hysteresis between formation and dissociation.

Sarkar introduced a set of parameters to characterise the progression of thermogelation. The first is the incipient precipitation temperature IPT, which is the temperature at which light transmission at 545 nm through the sample is reduced to 95.7% of its original value. The second point to be reached is the cloud point, CP, at which light transmission is reduced to 50% of its original value. The gels are translucent rather than transparent and the loss of light transmission which can be observed visually at the CP is a measure of thermogelation of the polymer in solution. The incipient gelation temperature, IGT, as indicated on the gelation curve, is the temperature of minimum viscosity which just proceeds gelation and the coinciding rise in viscosity.

As mentioned previously, the presence of unsubstituted regions plays a vital role in solubility and gelation. The viscosity of a solution can be increased at lower temperatures by residual crystalline components of the initial cellulose, and at higher temperatures by association, aggregation and, perhaps, crystallisation of the more highly substituted regions (Greminger and Savage, 1973). The nature and proportion of the substituting group have a great effect upon the gelling characteristics of the polymer.

Sarkar (1979) has investigated the effect of substitution and molecular weight on IPT and CP as shown in Table 1.2. He found that the decrease in hydrophilic character with an increase in MS correlates well with a decrease in CP. There was no observed effect on IPT attributable to an increase in molecular weight. He interpreted this as being due to the very wide molecular weight distribution within each sample. The IPT was interpreted as reflecting the precipitation of the high molecular weight fraction which was present at similar levels in all the polymer samples studied. However, the cloud point, which is not

thought to be greatly affected by the very high molecular weight fractions, as they have already precipitated-out by this point, showed a decrease with increasing molecular weight for the HPMC samples and little change for MC. Sarkar concluded that it is the methoxyl substitution that is primarily responsible for gelation and the hydroxypropyl substitution that is responsible for altering the gelation characteristics. The CP decreases in the order K>F>E (Table 1.1) reflecting an increase in the total degree of substitution and in the hydroxypropyl substitution, with all the CP values being larger than those observed for MC samples.

Sarkar (1979) also investigated the effect of hydroxypropyl molar substitution on gel strength. He found that the gel strength was drastically reduced for a 2% solution of DS 1.63-1.88 on increasing the hydroxypropyl MS from 0 to 0.2. The effect of both methoxyl and hydroxypropyl substitution is to increase the hydrophobicity of the polymer. However, on increasing hydroxypropyl substitution of MC the gelation temperature is increased and the gel strength is decreased. He concludes that this can be due only to steric factors. On maintaining the hydroxypropyl substitution but decreasing the total degree of substitution by lowering the methoxyl content, the same characteristic effects are observed, but this time due to a decrease in the hydrophobicity of the polymer.

1.4 Origin of Hydrophobic Association

There are two factors that can drive a system from one state to another:

1) an increase in entropy (positive ΔS)

2) a decrease in enthalpy (negative ΔH)

The simplest situation is when both factors pull in the same direction. For example, dissolving a salt in water will increase the entropy (by increasing the number of independently-moving species), and if the dissolving process is also exothermic (negative ΔH, because the system is losing energy) then the solution will remain stable at all temperatures.

$$\Delta G = \Delta H - T\Delta S$$

If both the driving factors ΔH and ΔS pull in opposite directions, ΔG, the free energy, can be either positive or negative depending on the temperature. This situation holds for melting of a typical hydrophilic polysaccharide, heat energy is applied to dissociate the junction zones (+ve ΔH) and there is a move from an ordered gelled network to a random solution due to an increase in conformational mobility (+ve ΔS). At the melting temperature the two factors cancel and the free energy is zero:

$$\Delta H = Tm\Delta S$$

At high temperatures $T\Delta S > \Delta H$, giving $\Delta G < 0$, so that the system shifts to the solution state. At lower temperatures $T\Delta S < \Delta H$ and $\Delta G > 0$, favouring the gel state.

As a starting point for explaining why hydrophobically-substituted polysaccharides should gel on heating rather than on cooling, it is useful to consider first the behaviour of simple hydrocarbons. Although their free energy is far higher in water than in most non-aqueous solvents, the enthalpy is lower (Tanford, 1980), i.e., hydrocarbon-water interactions are more favourable than hydrocarbon-hydrocarbon, so that in this sense 'hydrophobic' is a misnomer. The increase in free energy must, therefore, come from a decrease in entropy, which is normally interpreted as distortion of the hydrogen-bonding in water to give highly constrained 'cage-like' structures around the foreign species (if these cannot themselves form hydrogen bonds with water). One piece of direct evidence in favour of this interpretation is that when aqueous solutions of hydrocarbons (or other non-polar species, notably inert gases) are cooled, they produce solid clathrates (hydrophobic hydrates) in which the guest species are trapped and free to rotate within a hydrogen-bonded cage of water molecules (Franks, 1983). The negative entropy arises from the reduction in the allowed degrees of freedom of water molecules in the vicinity of the apolar molecule.

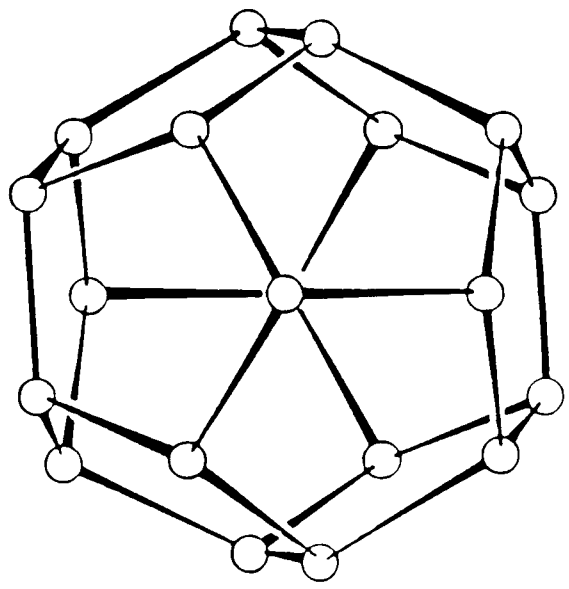


Figure 1.8 Typical clathrate geometry. Circles represent oxygen atoms and the lines are hydrogen bonds (From Franks, 1983).

Extrapolating this concept to apolar substituents on polymers, the solution is now the low-entropy, low-enthalpy state (the exact opposite to the conventional hydrophilic polysaccharides). Raising the temperature increases the relative importance of the entropy term until it becomes the dominating factor, and therefore promotes gelation. Although it is enthalpically favourable for the formation of hydrophobic hydrates, it is entropically very unfavourable as it is imposing a degree of strain, and the ordered arrangement becomes increasingly less stable on heating. Eventually a temperature is reached where the apolar residues are withdrawn from aqueous contact and the ordered water is released, causing an increase in entropy. The exposed hydrophobic groups unable to bind with water molecules are forced into associations with other hydrophobic groups of neighbouring molecules, resulting in gelation, however, this is enthalpically costly. Franks (1975) concludes that the hydrophobic interaction is a partial reversal of the entropically unfavourable hydration process, where hydrophobic interaction is seen as an increase in the order of water rather than the interaction between non-polar groups. The process is therefore, entropically driven, not enthalpically driven.

It should be noted, however, that although both enthalpy and entropy have opposite sign in 'hydrophobic' and 'hydrophilic' gelation, their direction of change with temperature is the same in both cases: entropy increases on heating, and enthalpy decreases. Indeed we could (simplistically) regard hydrophobic gelation as a 'melting' process (disruption of water 'cages'). Endothermic DSC peaks (Section 2.4) on heating, and exothermic on cooling, are therefore to be expected for either hydrophobic interactions or 'junction zones'.

For amphiphilic polymers, where both modes of interchain association may be involved, it seems likely that the overall behaviour will represent a complex interplay of hydrophobic and enthalpic interactions. This is the situation addressed in the present work.

Table 1.1 Methocel products offered by the Dow Chemical Company in premium grades.

Product	Viscosity mPa-S[a]	DS methoxyl	MS hydroxypropyl
A 15LV	15	1.6—1.9	0
A 4C	100	1.6—1.9	0
A 4M	4,000	1.6—1.9	0
E 5LV	5	1.8—2.0	0.20—0.31
E 15LV	15	1.8—2.0	0.20—0.31
E 50LV	50	1.8—2.0	0.20—0.31
E 4M	4,000	1.8—2.0	0.20—0.31
F 50LV	50	1.7—1.9	0.10—0.20
F 4M	4,000	1.7—1.9	0.10—0.20
K 3	3	1.1—1.6	0.10—0.20
K 35	35	1.1—1.6	0.10—0.30
K 100LV	100	1.1—1.6	0.10—0.30
K 4M	4,000	1.1—1.6	0.10—0.30
K 15M	15,000	1.1—1.6	0.10—0.30
K 100M	100,000	1.1—1.6	0.10—0.30

[a] As a 2% aqueous solution.

Table 1.2 Precipitation and cloud point temperatures of 2% aqueous solutions as a function of molecular weight and substitution. (From Sarkar, 1979)

	IPT°C	CP°C	MS	DS
A15	43	63		
A27	47	62	0	1.6-1.8
A400	47	62		
A4M	48	61		
E15	61	67		
E50	61	64	0.2-0.3	1.65-1.9
E4M	58	61		
F50	61	69		
F4M	60	65	0.1-0.2	1.6-1.8
K35	61	79		
K100	60	76	0.1-0.3	1.1-1.4
K4M	61	70		

Chapter 2: INVESTIGATIVE TECHNIQUES

2.1 Introduction

There are a number of techniques available for the characterisation of molecular conformation and association in polysaccharide systems. Chiroptical techniques offer a direct route to conformational information since the interaction of polarised radiation with dissymetric molecules is critically sensitive to stereochemical organisation. Both optical rotation (OR) and circular dichroism (CD) are based on this principle and allow conformational transitions during gelation to be monitored.

Energy changes associated with gelling and melting are conveniently measured using differential scanning calorimetry giving a direct measure of the enthalpy change for the underlying conformational transition.

Viscosity measurements of large macromolecules in solution give a sensitive indication of the size of the individual polymer units, and can be used to monitor any change in molecular dimensions in response to changes in solvent environment.

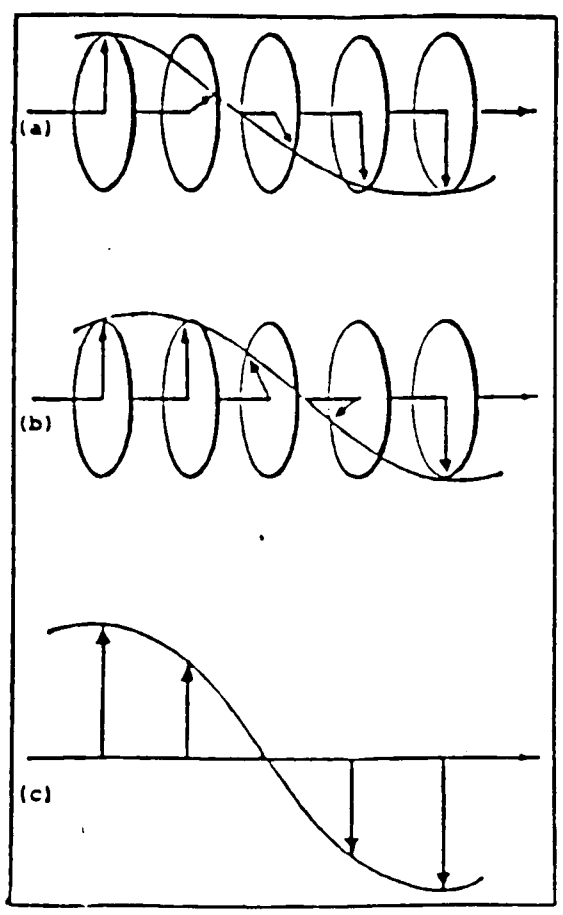
Rheological testing by application of non-destructive oscillatory shear (mechanical spectroscopy) gives a convenient index of gel strength, and the process of gelation and melting can be monitored by changes in the structural moduli of the system. By varying the frequency or the strain of the applied oscillation, the strength and timescale of molecular interaction can also be characterised.

Each of these techniques is discussed here in more detail with reference to the experiments carried out in this study.

2.2 Optical Rotation

The response of asymmetric molecules to polarised light is characterised by the techniques of optical rotation and circular dichroism. We shall consider firstly OR, which is a measure of the differential refraction of left and right circularly polarised light.

Light is a transverse electromagnetic wave with the beam being made up of magnetic and electronic components at right angles to each other oscillating perpendicularly to the axis of propagation. Plane polarised light can be considered as being composed of two identical circularly polarised components of half amplitude which are rotating in opposite directions at equal angular speed.



a)Right-hand circularly polarised light

b)Left-hand circularly polarised light

c)Plane polarised light: resultant of a) and b)

Figure 2.1 Polarised light.

If plane polarised radiation of any given wavelength is passed through an optically active substance (i.e., one containing dissymetric molecules) the plane of polarisation will be rotated.

In optically active material the refractive index differs for the two component left and right polarisations, consequently their speed through the material differs. If the substance has a higher refractive index for left rather than right circularly polarised light, then the left-hand component will be retarded more. Because of the differential retardation of the component waves, the plane of polarisation of the resultant beam is tilted from the original plane of polarisation.

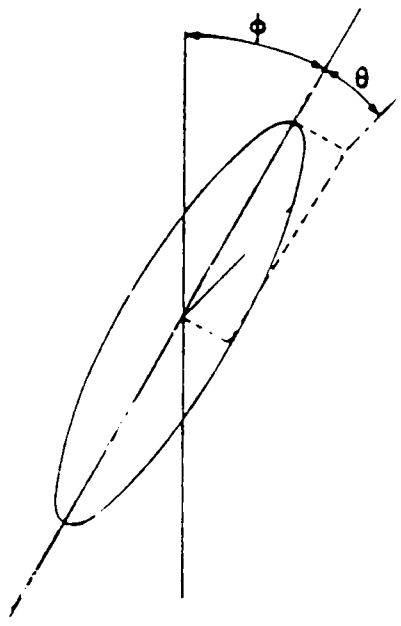


Figure 2.2 Angle of rotation (From Morris and Frangou, 1981).

The degree of rotation, is determined by the nature of the optically active dissymetric molecule. The rotation may by be either clockwise, dextrorotatory (+ve), or anti-clockwise, levorotatory (-ve). OR is usually expressed in terms of molar rotation [ϕ] with units deg cm /dmol;

$$[\phi] = \phi m/cd$$

where ϕ is the measured rotation, m is the molecular weight, c is the concentration and d the path-length through the sample.

Since optical rotation is critically dependant on molecular geometry, it provides a convenient method for following the conformational changes associated with formation and melting of polysaccharide gels (Rees *et al.*, 1982).

2.3 Circular Dichroism

The principles of circular dichroism, CD, are closely allied to those of OR as it also concerns the passage of electromagnetic radiation through an optically active substance.

Whereas OR results from a difference in refractive index for left and right circularly polarised light, CD results from a difference in absorption of these two components. CD is sensitive to changes in the stereochemical organisation of the polymer and the chiroptical properties vary with changes in the asymmetry of the chromophore environment due to intramolecular (conformational change) and intermolecular interactions (association).

On passing electromagnetic radiation consisting of two circularly polarised beams through an optically active substance, in the region of an absorption band, where the wavelength of the radiation supplied matches the energy requirements for promotion of electrons from the normal ground state to an excited higher energy level, one component will be preferentially absorbed in addition to the preferential retardation of either the left or right hand components as discussed for OR. The consequence of this is that the two circular oscillations can no longer be combined into a linear oscillation and the light now becomes elliptically polarised (Figure 2.2). The eccentricity of the ellipse obviously depends upon the differential absorption of the left and right-hand components. CD may therefore be defined as the angle of ellipticity, (θ), where tan θ is equal to the ratio of the minor axis of the ellipse to the major axis.

Just as OR is expressed in molar rotation, so CD can be expressed in terms of molar ellipticity, [θ].

CD bands may be either positive or negative depending on whether left or right circularly polarised light is preferentially absorbed. Both the sign and the intensity of each CD band are sensitive functions of molecular structure. Because CD relies upon the differential absorption of energy by a chromophore it obviously occurs within a very narrow wavelength range and falls off rapidly on moving away from the absorption band centre. The equipment used determines the range of the spectrum obtained as it is confined to the chromophores which absorb within the accessible wavelength of the instrument. The lower wavelength limit of most current commercial apparatus is approximately 185 nm.

2.4 Differential Scanning Calorimetry

Differential scanning calorimetry, DSC, is a thermal analytical technique in which a physical property of a substance and/or its reaction products are measured as the difference in energy exchange between the sample and a reference material as a function of temperature whilst the sample is subjected to a controlled temperature programme. All substances undergo either physical or chemical changes when heat energy is applied. These may be phase transitions (e.g. ice/water), conformational state transitions (e.g. order/disorder) and mass or compositional changes (e.g. oxidation) (Wright, 1984).

In its application to gelling polysaccharides, DSC is used mainly to measure the absorption or release of heat energy as the sample undergoes conformational transitions during heating and cooling at a constant rate. However, any modifications or alteration to the structure of the molecule that effects the thermal stability of that molecule can, in principle, be discerned by DSC. Information can also be gained on biopolymer interactions, rates of reaction or process kinetics, composition and state, manifested by an increase or decrease in temperature in the form of a thermogram depicting differential heat flow (ordinate) against temperature (abscissa). In the absence of any transition a monotonic line is obtained; any ordinate deflection represents an absorption or evolution of heat by the sample i.e. an increase or decrease in the enthalpy. The nature of DSC measurement is such that it is not the actual amount of heat absorbed or evolved that is measured, but the excess power input required to heat or cool the sample to maintain the same temperature as the reference material. The heat capacity of the reference material should balance that of the sample in temperature ranges where no transition is occurring.

An idealised thermogram for an endothermic conformational transition is depicted in Figure 2.3.

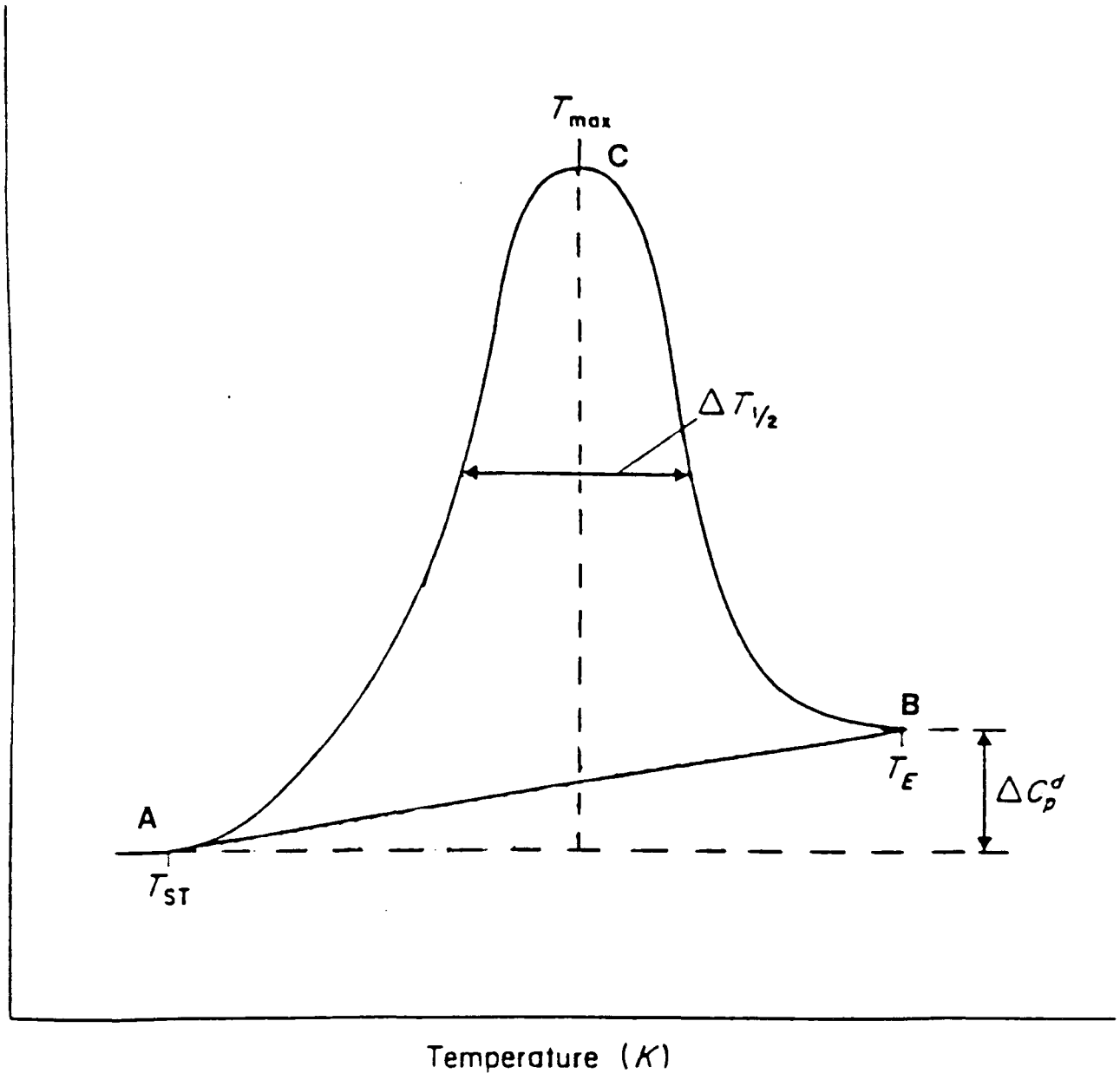


Figure 2.3 Idealised thermogram of an enthalpic transition.

The enthalpy of the transition is calculated directly from the area of the peak ABC using a base line that would be the line recorded in the absence of any transition. Difficulties in determining the position of the base line result from differences in heat capacity between the reference material and the sample and also because heat transfer between substance and container may change considerably in the region of the transition (Hemminger and Höhne, 1984). In this study the equipment used is fully automated and baselines and enthalpies are calculated by computer integration.

The relationship between enthalpy and peak area can be established as follows providing the ordinate is calibrated in terms of heat capacity:

$$d\Delta H / dT = \Delta Cp$$

Ideally a flat baseline either side of the transition should be obtained, the value of the ordinate giving a measure of the heat capacity of the sample relative to that of the reference.

The position of the transition peak along the profile gives a direct measure of the transition temperature. T_{MAX}, the temperature at maximum heat flow into the sample is the transition temperature most often quoted. The temperature of the onset can also be obtained by constructing a tangent to the leading edge of the DSC peak and taking the temperature at the point of intersection with the baseline.

The peak width at half peak height, $\Delta T½$ is a measure of the co-operativity of the molecular transition. The smaller the value the more co-operative the process. If all molecular rearrangements occur simultaneously then the peak of the transition will be very sharp and narrow indicating a high level of co-operativity.

2.5 Viscosity

Solution viscosity provides a measure of the size of polymer molecules and as such can be used as an empirical measure of molecular weight. Changes in viscosity of a solution result from the introduction of relatively large solute molecules into an environment of smaller solvent molecules. Viscosity is defined as the resistance of a fluid to flow and is obviously dependent upon the nature of both the solvent and the solute. In order to understand the principle of viscosity and its measurement it is first necessary to examine the fundamental mechanics of viscous flow . In Figure 2.4 we consider a Newtonian (ideal) liquid held between two infinitely large parallel plates. One of these is at rest; the other is displaced laterally with a constant velocity.

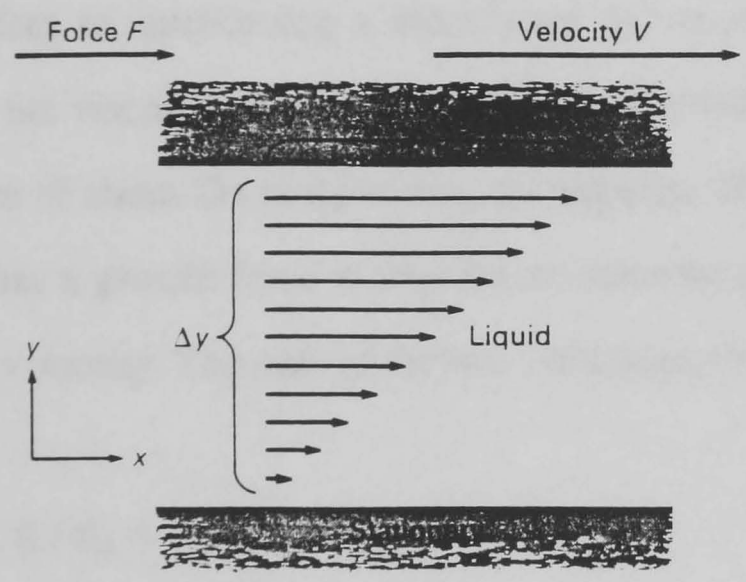


Figure 2.4 Shearing of a Newtonian liquid between parallel plates (From Van Holde, 1985).

A velocity gradient is created within the bulk of the liquid, with that adjacent to the moving surface having an appreciably higher velocity than that next to the stationary surface. The ratio of the horizontal movement (Δx) to the distance between the plates (Δy) is termed the shear strain γ. The strain developed per unit time is defined as;

$$\dot{\gamma} = d\gamma / dt$$

where $\dot{\gamma}$ is termed the rate of strain or the shear rate. The force per unit cross-sectional area that is applied to the liquid is the shear stress σ. It is this relationship between shear stress and shear strain for Newtonian liquids which characterises the resistance to flow and hence the viscosity (η) of the liquid.

$$\eta = \sigma / \dot{\gamma}$$

To determine the effect of introducing a biopolymer solute into a solvent it is first necessary to measure the viscosity of the solvent alone by determining the force required to produce a given rate of shear. On re-measuring the viscosity after the addition of solute particles it is found that a greater force is required to maintain the same shear rate, i.e., there is an increase in viscosity. The ratio of the two viscosities yields;

$$\eta / \eta_S = 1 + \nu\phi$$

where η is solution viscosity, η_S is solvent viscosity, ϕ is the volume fraction of the solute molecules and ν is a numerical constant whose value has been previously calculated for various shapes of macromolecules.

The greater force required for deformation is due to the smaller volume of fluid in which the overall deformation must take place as the solute particles have limited deformation yet they occupy a fraction of the total volume of the fluid (hence the relationship between viscosity and volume fraction of solute). This applies only to dilute solutions where the solute particles are considered as individual moieties and does not take into account concentration effects and solute-solute interactions.

The ratio of solution viscosity to solvent viscosity is termed the relative viscosity, η_{rel};

$$\eta_{rel} = \eta / \eta_S$$

It is more convenient to express data in terms of the specific viscosity where unity is subtracted from relative viscosity;

$$\eta_{sp} = \eta_{rel} - 1 = (\eta - \eta_S) / \eta_S$$

This is a measure of the fractional change in viscosity produced by adding the solute. Considering a macromolecular solution to be equivalent to a suspension of particles within the solvent, it is obviously proportional to the concentration of the solute c. The ratio gives the reduced viscosity, η_{red};

$$\eta_{red} = \eta_{sp} / c$$

In order to eliminate the interaction effects and consider each polymer coil being sufficiently distanced from its neighbour so as not to interfere with the flow of surrounding polymers, the reduced viscosity is extrapolated to zero concentration. The limit of $\eta sp/c$

as $c \rightarrow 0$ is termed the intrinsic viscosity, $[\eta]$, and depends only upon the properties of isolated molecules.

If the polymer is charged the external solvent environment greatly influences the effective radius, i.e., molecular volume, of the molecule. Changes in pH or ionic strength will cause the coil to either shrink or expand and hence alter the viscosity of the solution. In practice this can be monitored by measuring viscosity at a set temperature at various ionic strengths and acidities and extrapolating to zero. This then gives an indication of the actual size of the individual polymer molecules and how they react to their environment.

Viscosity can be measured in a number of ways, the most common method used is the simple Ostwald Capillary Viscometer. Viscosity is extremely temperature dependent and Ostwald viscometry is carried out in a thermostatted water bath to maintain the desired temperature.

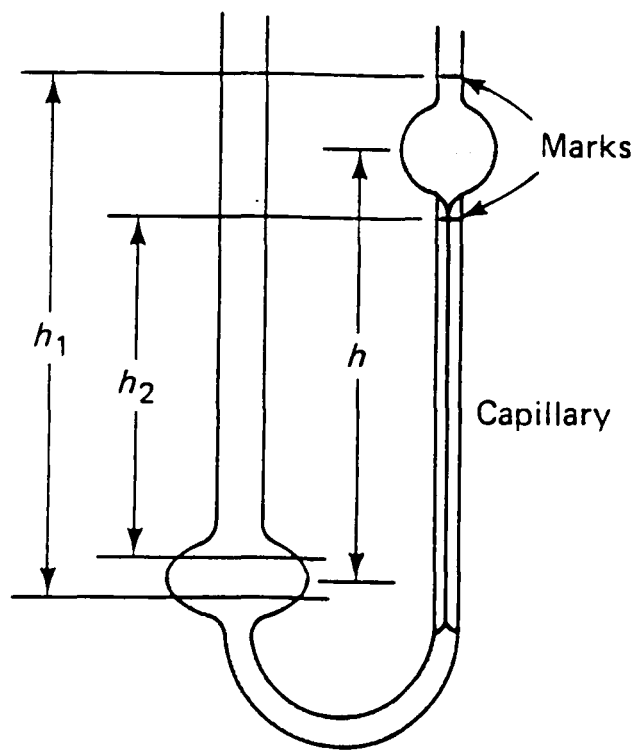


Figure 2.5 Ostwald viscometer (From Van Holde, 1985).

The time taken for a given volume of liquid, as measured by marks on the upper bulb, to flow through a capillary is measured and related directly to the viscosity.

$$\eta / \eta_S = (t / t_S) * \rho / \rho_S$$

where ρ is solution density and ρ_S solvent density, t is time for known volume of solution to flow through a pre-determined distance and t_S is the flow time of the solvent.

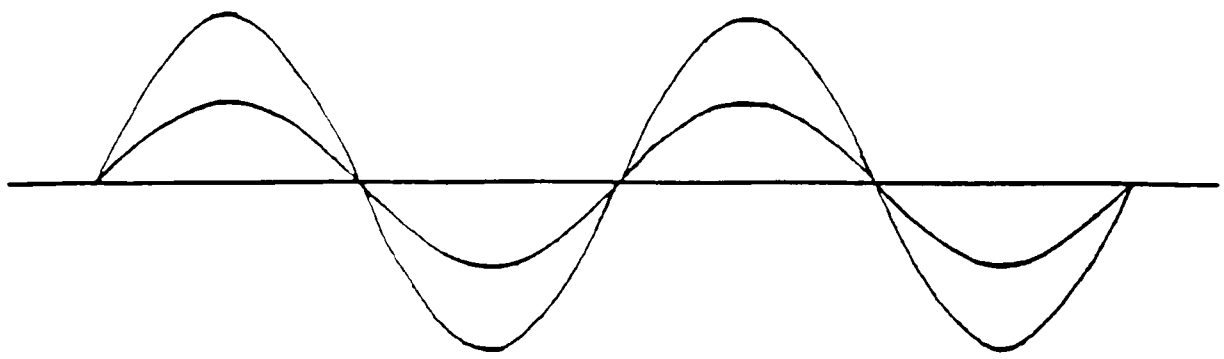
For polysaccharides of very low concentrations (<4 mg/ml) $\rho \equiv \rho_S$ and hence we can approximate the relative viscosity to t / t_S.

Velocity gradients are set up within the liquid flowing through the capillary. The velocity of the liquid will be near zero at the walls, which are stationary, and will be at a maximum at the centre of the capillary; hence, large shear rates are generated within the narrow capillary, which cannot easily be changed or precisely specified and this apparatus cannot be used with any degree of accuracy for investigation of shear-rate dependence. It does, however, provide a convenient, and highly sensitive, method of detecting changes in molecular dimensions.

2.6 Mechanical Spectroscopy

Mechanical spectroscopy is concerned with the characterisation of a material, for example a gel or concentrated hydrocolloid solution, by monitoring its response to low-amplitude oscillatory shear.

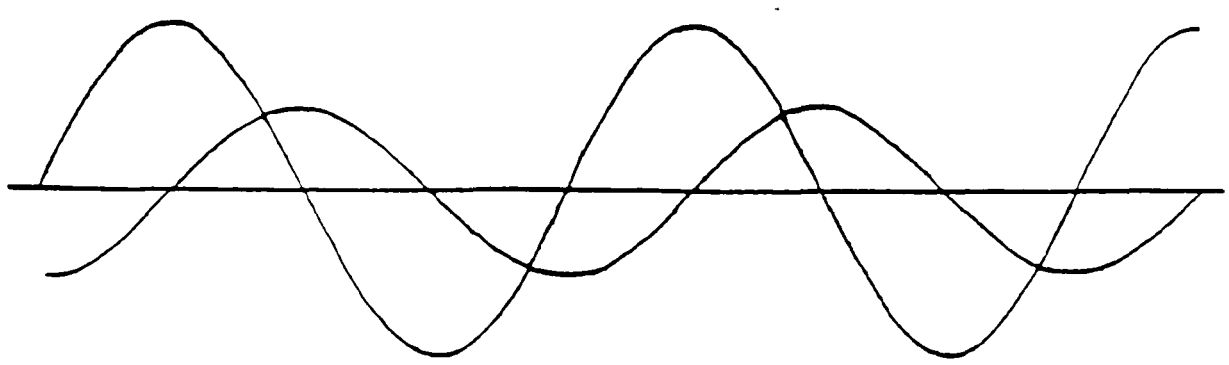
Gels are rheologically complicated structures whose component features can be more easily understood in terms of two idealised extremes: perfectly elastic solids and perfect Newtonian liquids. The former are characterised by the stress generated being proportional to the strain at any one point within the cycle, whereas for Newtonian liquids the stress developed is proportional to the rate of strain. For elastic solids the energy required to deform the sample is stored and there is complete recovery when the stress is removed. The stress produced is exactly in-phase with the imposed strain, with maximum stress at the extremes of the oscillatory cycle. The ratio of stress to strain gives a measure of the elastic or 'storage' modulus of the sample, G'.



$$G' = \frac{\text{In-phase stress}}{\text{Applied strain}}$$

Figure 2.6 The elastic modulus, G'.

For a Newtonian liquid the energy used in deforming the sample is lost as heat. The stress generated is at a maximum at the mid-point of the cycle, where the rate of strain is greatest, and falls to zero at the extremes, 90° out of phase with the applied strain. The ratio of stress to the rate of strain provides a measure of the viscous or 'loss' modulus, G".



$$G'' = \frac{\text{90° Out-of-phase stress}}{\text{Applied strain}}$$

Figure 2.7 The viscous modulus, G".

The response of real samples can be separated into the in-phase component and the out-of-phase component to give individual values of G' and G", and the overall response is characterised by the unresolved complex modulus, G* ;

$$G^* = (G'^2 + G''^2)^{½}$$

Taking into account the frequency of the applied oscillation (ω), we obtain the dynamic viscosity, η*

$$\eta^* = G^* / \omega$$

The 'phase-lag', δ, between the applied deformation (strain) and the resistance generated (stress), which, as described above, ranges from 0 for a perfect solid to 90° for a perfect liquid, gives a measure of the ratio of liquid-like character to solid-like character of the system, with:

$$\tan \delta = G'' / G'$$

Because small-deformation mechanical spectroscopy can be applied to both solids and liquids, it can conveniently be used to monitor gel formation and melting during thermal cycling. The importance of having only a small deformation is that the sample remains relatively unperturbed and delicate gel structures are not disrupted.

By subjecting a sample to a constant strain and varying the frequency the mechanical spectrum of the sample at any particular temperature can be monitored. Figure 2.8 shows the typical spectra obtained from: a) a dilute solution, b) a gel, c) a concentrated solution.

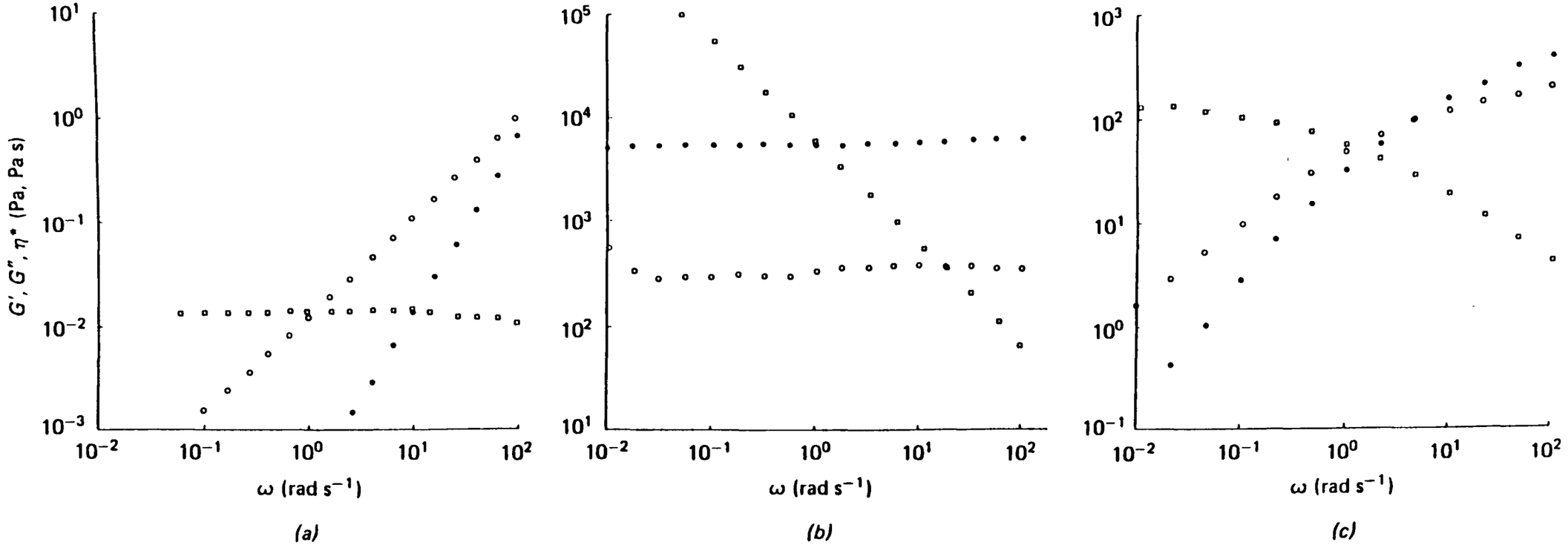


Figure 2.8 Typical mechanical spectra for polysaccharide systems showing the frequency dependence of (●) G' and (○) G" (in Pa) and (□) η* (in Pa s) for (a) dilute solution; (b) a gel; and (c) a concentrated solution. (From Morris and Ross-Murphy, 1981).

Chapter 3: MATERIALS AND METHODS

3.1 Pectin

The high methoxy pectin used was HP Bulmers rapid set, sodium form (Batch number c8/108).

The same sample was used for the esterification procedure to produce very high methoxy pectin.

Esterification of high methoxy pectin

Methanolic sulphuric acid was prepared by the addition of concentrated sulphuric acid to methanol to give a 1M concentration solvent. The acid was added very slowly to the methanol with mechanical stirring in an acetone/dry ice bath. High methoxy pectin was added sufficient to give a 1% solution. This was stirred for 3 days in a cold room at 1°C. The pectin was recovered by vacuum filtration and washed with 60/40 v/v ethanol/water until the washings were neutral. It was then further washed with excess absolute alcohol and ether and left to dry.

Pectin sample preparation for OR, CD and mechanical spectroscopy measurements

All the samples were prepared in the same way for both high methoxy and very high methoxy pectin. Due to the thermo-irreversibility of the gels, each sample was prepared individually as required for each experiment and maintained at a high temperature (above 70°C) before use.

A 6% stock solution of both pectin types was prepared by dissolving the pectin in hot water and stirring for approximately 1 hour. The solution was neutralised to pH7 with 1M sodium hydroxide. The pectin stock solution, water, and ethylene glycol were heated to 70°C and added together in the proportions shown in Table 3.1. One drop of hydrochloric acid was added to each solution to reduce the pH below 2. The resulting solutions contained 1% pectin. The samples were loaded hot into the CD and OR cells and also onto the flat plate of the rheometer.

Optical rotation measurements

OR measurements were carried out on a Perkin-Elmer 241 polarimeter using a 1cm quartz thermostatted cell at a wavelength of 365 nm. The cell temperature was controlled using a Haake F3 refrigerated circulating water bath.

The samples were loaded at 95°C into the cells and cooled at 5 degree intervals allowing sufficient time for the sample temperature to equilibrate. The first cooling was from 95°C to 5°C, followed by heating to 95°C and a second cooling back to 5°C. The OR data were plotted directly against temperature.

CD measurements

These measurements were carried out on a Jobin Yvon Auto-dichrograph Mark V, using a 1mm thermostatted cell and scanning from 273 to 190 nm with a 1 second integration period. The cell temperature was controlled using a Haake F3 refrigerated circulating water bath and measured by a thermocouple inserted into the neck of the cell. The

samples were loaded hot at 95°C and cooled with a 15 degree interval to 5°C allowing time for equilibration prior to each reading. This was followed by a heating regime to 95°C and a subsequent second cooling to 5°C.

The ellipticity was calculated using the formula;

$$[\theta] = \theta m / cl$$

where θ is the maximum ellipticity measured in degrees, m is the molar mass, which was taken as 208 for the fully esterified very high methoxy pectin sample and 211 for the high methoxy sample (~ 70% esterification). The concentration was 10 mg/ml and the path cell length 1mm. The ellipticity values were plotted directly against temperature.

Mechanical Spectroscopy

These measurements were carried out using a highly sensitive small deformation prototype rheometer developed and built by Dr. R.K. Richardson at Silsoe College (PhD Thesis, 1991). It is a fully automated, computer controlled system allowing programmable temperature and frequency sweeps to be made. The machine incorporates a temperature controllable base attached to a Haake F4 programmable refrigerated water bath, with cone and plate geometry of cone angle 0.05 rad and radius 25mm.

The samples were degassed under vacuum and loaded hot onto the plate pre-set at 90°C and the gap between cone and plate was sealed with a light silicone oil (Dow Corning 200/1000cs) to prevent water loss. The samples were cooled at a rate of 1°/min with readings every minute down to 5°C. The strain was programmed at 2% and the frequency

at 10 rad/sec. The samples were subsequently heated and re-cooled under identical conditions. All data was stored automatically on computer and used for plotting mechanical spectra.

Ostwald viscometry

A Griffin BS UA No. 4769 glass viscometer was used, suspended in a thermostattically controlled water bath (Townson and Mercer, Series II), at 25°C. A 2% stock solution was made using both pectin types as before and neutralised with 1M sodium hydroxide. A 4M sodium chloride solution was prepared which, when diluted in the sample, gave a 0.5M salt concentration. The samples were prepared as shown in Table 3.2.

The resulting pectin concentration in each sample was 0.3%. The flow time of the sample was measured using a stop-watch to an accuracy of 1/100th of a second. This was repeated until a steady flow time was obtained. Distilled water was used as the solvent to calculate relative viscosities.

3.2 Cellulose Derivatives

Two cellulose derivatives were used, supplied by the Dow Chemical Company. The chemical composition of the samples (N. Sarkar, personal communication) is shown in Table 3.3.

Table 3.3 Analytical data for K4M and A4M.

sample	METHOCEL K4M MM90092303K	METHOCEL A4M MM88051912A
2% solution viscosity mPas	4547	4969
% methoxyl	22.3	29.99
DS	1.4	1.81
% hydroxypropyl	8.5	0
MS	0.22	0
% sodium chloride	0.63	0.56

DS = Degree of methoxyl substitution, MS = Molar substitution of hydroxypropyl group.

Preparation of cellulose ether samples for DSC and mechanical spectroscopy

A 3% stock was made of each of the cellulose derivatives by dissolving in one third of the required water at 70°C to ensure complete dispersion. The other two thirds of water was added cold and the solution stirred for a minimum of 20 minutes. A 50% solution of sucrose was prepared from Tate and Lyle granulated sugar. The samples were prepared as shown in Table 3.4 for both co-solutes; ethylene glycol and sucrose. The samples were exhaustively de-gassed under vacuum before use.

DSC measurements

These were carried out on a fully automated and programmable Setaram micro DSC calorimeter. The sample cells were loaded with approximately 0.9g of sample and the reference cells were filled with the same mass of water to an accuracy within 0.01g. Heating and cooling scans were carried out at 0.2, 0.5 and 0.8 degrees per minute between 95°C and 5°C. Thermograms of heat flow against temperature were produced, baselines fitted and enthalpies calculated.

Mechanical Spectroscopy

This was carried out on the same machine as described previously, but the samples were loaded cold, heated and then re-cooled. Programmed temperature scans were made at a rate of 1°/minute with frequencies of 1 and 10 rad/sec, and frequency sweeps were made at set temperatures, with frequency ranging from 0.1 to 101 rad/sec at 2% strain.

Table 3.1 Preparation of high methoxy and very high methoxy pectin samples

mls of stock	mls of water	mls of ethylene glycol	% of ethylene glycol
2.5	0.5	12.0	80
2.5	3.5	9.0	60
2.5	4.5	8.0	53.3
2.5	5.0	7.5	50
2.5	6.5	6.0	40
2.5	7.5	5.0	33.3
2.5	8.0	4.5	30
2.5	8.5	4.0	26.7
2.5	9.5	3.0	20

Table 3.2 Preparation of samples for viscometry

mls of pectin stock	mls of water	drops of HCL	pH	mls of 4M NaCl
3.0	17.0	-	7	-
3.0	17.0	1	2	-
3.0	14.5	-	7	2.5
3.0	14.5	1	2	2.5

Table 3.4 Preparation of etherified cellulose samples

mls of stock	mls of water	mls of ethylene glycol or 50% sucrose	% of ethylene glycol	% of sucrose (where applicable)
5	15.0	0	0	0
5	14.0	1.0	5	
5	13.0	2.0	10	5
5	12.0	3.0	15	
5	11.6	3.4	17	
5	11.2	3.8	19	
5	11.0	4.0	20	10
5	10.0	5.0	25	
5	9.6	5.4	27	
5	9.0	6.0	30	15
5	8.0	7.0	35	
5	7.0	8.0	40	

Chapter 4: RESULTS AND DISCUSSION OF PECTINS

4.1 Introduction

As outlined in Chapter 1, suppression of charge to allow pectin chains to associate into a gel network can be achieved in two different ways. Samples with a low degree of methyl-esterification can be gelled by the addition of calcium ions, which balance the charge of pectate carboxyl groups within ordered 'egg-box' junctions (Section 1.2.4). Gelation of pectins with a higher ester content can be achieved by lowering the pH, to suppress the charge of unesterified carboxyl groups, and introducing high concentrations of a co-solute, such as sucrose or ethylene glycol (Section 1.2.5).

The normal interpretation of this latter mode of gelation is that, when chains are virtually uncharged, the reduction in water activity due to the presence of the co-solute is sufficient to promote the formation of chain-chain aggregates which act as the 'junction zones' of the gels. However, in a study of the effect of ester content on pectin gelation at fixed pH (1.9) and fixed concentration of ethylene glycol (60% w/v), Morris *et al.* (1980) found that gel strength increased with increasing ester content (as expected) up to a degree of esterification (DE) of about 72%, but on further increasing the DE, the gels became progressively weaker, with complete loss of gelation by DE≈95%. Changes in circular dichroism (Section 2.3) found to accompany gel formation at low esterification levels were still observed in the non-gelling sample, and indeed, were of greater magnitude. One of the main objectives of the present research has been to verify this puzzling behaviour, and to explore its molecular origin.

Two pectin samples have been used: one with a DE close to that at which optimum gelation was observed in the previous investigation (~70%), and a chemically esterified sample (Section 3.1) with a DE close to 100%. These will be referred to as "high methoxy pectin" (HM) and "very high methoxy pectin" (VHM) respectively. Gel strength has been characterised by non-destructive oscillatory measurements (Section 2.6) rather than by the more empirical compression testing used by Morris *et al.* (1980). Low amplitude oscillatory

measurements have also been used to monitor the temperature-course of gelation and the thermal stability of the resulting gels. The associated changes in chiroptical properties (diagnostic of changes in chain conformation) have been characterised by optical rotation (Section 2.2) as well as by circular dichroism, the approach used previously (Morris *et al.*, 1980).

The most informative extension to the earlier work, however, has been to explore the effect of changes in the concentration of the co-solute ethylene glycol. As detailed below, the response to co-solute concentration gives clear indications of the nature of the intermolecular interactions responsible for network formation.

4.2 Circular Dichroism

The technique of circular dichroism is used to explore the conformational changes accompanying the sol-gel transition during thermal cycling. Changes in the nature of the solvent surrounding the relatively hydrophilic pectin molecules (very high methoxy pectin, which is almost fully esterified, has a ratio of polar OH groups to non-polar methyl groups, close to 2:1. High methoxy pectin, with a degree of esterification approximately 70%, has a considerably higher ratio of 2:0.7), would be expected to modify the behaviour of chain ordering and subsequent gelation.

Pectin samples were made from a stock solution as detailed in Section 3.1, and maintained at high temperature (80-90°C) prior to loading into the heated CD cells as gelation is found to be irreversible on heating. The samples were subjected to a cooling regime from 90 to 5°C, followed by heating to 90°C and a second cooling back to 5°C.

Figure 4.1 shows the circular dichroism (CD) spectra observed for high methoxy pectin in 60% ethylene glycol at temperatures spanning the range of the sol-gel transition on cooling. All the samples studied exhibited a similar positive change in ellipticity with a maximum in the region 200-215 nm. Figure 4.2a shows that there is a substantial increase in ellipticity on cooling which occurs as a comparatively sharp process as reported previously (Morris *et al.*, 1980). Similar changes, but of a somewhat greater magnitude, are observed (Figure 4.2b) for very high methoxy pectin. This intensification of CD is the expected (Rees *et al.*, 1982) response for a co-operative transition from a disordered 'coil' state to an ordered conformation due to a restriction in chain flexibility and an increase in average molecular dissymmetry.

As shown in Figure 4.3, the onset of the sharp increase in CD for both samples moves to progressively higher temperatures with increasing concentration of ethylene glycol, but the values obtained for very high methoxy pectin are systematically higher (by about 20°C).

There is also some indication of a decrease in the overall amplitude of the CD change at low concentrations of co-solute (below about 40% ethylene glycol).

As observed previously, the CD change is largely irreversible on heating, but there is evidence of a slight decrease in ellipticity at high temperature (Figures 4.4 and 4.5) with the values on subsequent re-cooling running below those on heating until the temperature range of the initial increase. The final ellipticity corresponds closely with that obtained after the first cooling. The hysteresis is considerably augmented for the very high methoxy pectin samples.

High methoxy pectin, from observation, forms a gel at all concentrations of ethylene glycol, including 60% as used previously. However, very high methoxy pectin does not form a gel visually with 60% ethylene glycol (or higher concentrations-80%) as reported before, but it does form a gel at lower concentrations. These results indicate a substantial degree of conformational chain ordering occurring in the non-gelling samples, it is therefore suggested that the loss of gelation is attributable to the subsequent packing of these ordered chains (Section 4.6) Further evidence for the loss of network coherence at high concentrations of ethylene glycol is provided in Section 4.4.

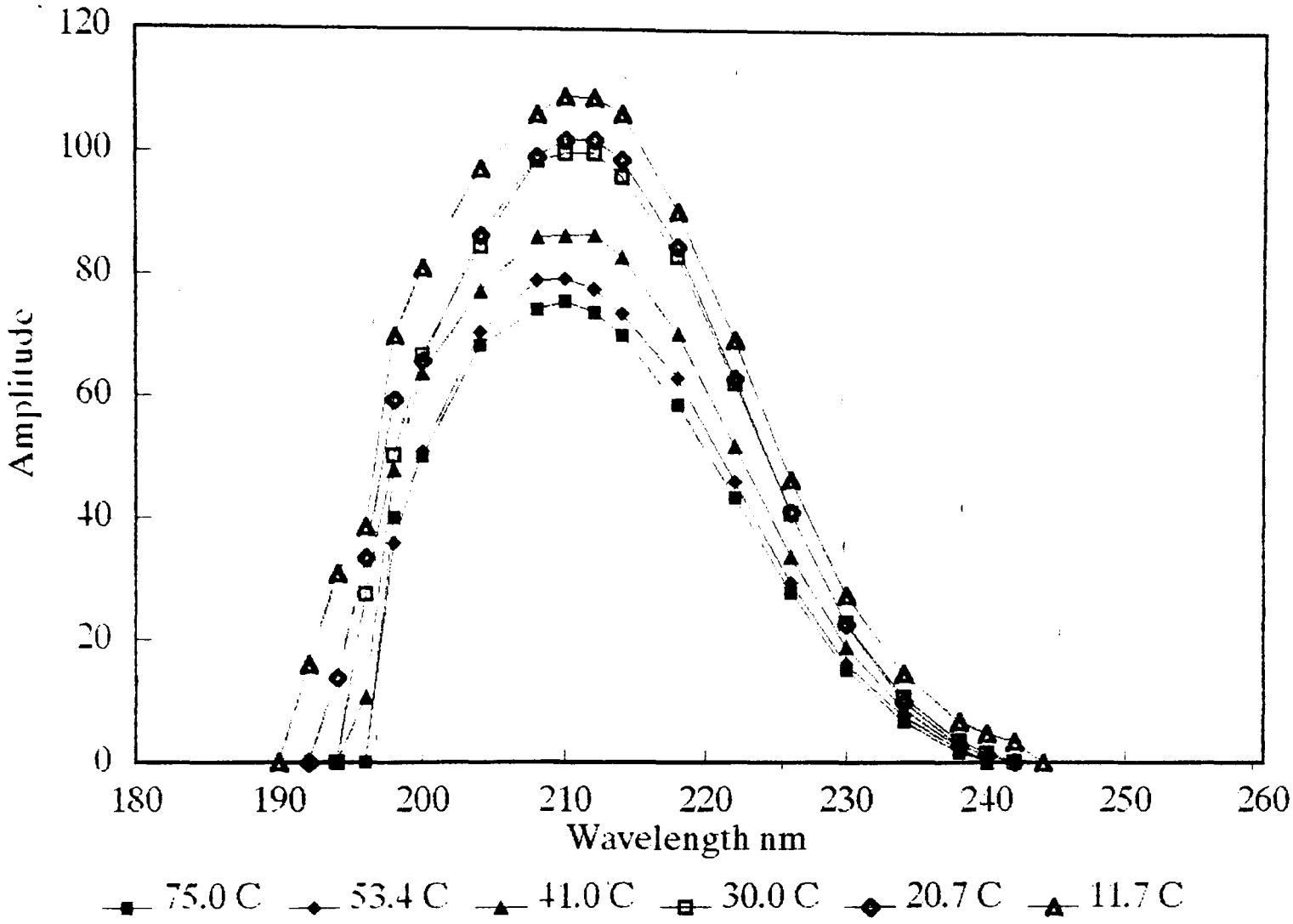


Figure 4.1 Circular dichroism of 1% high methoxy pectin with 60% ethylene glycol on first cooling. 1mm path length.

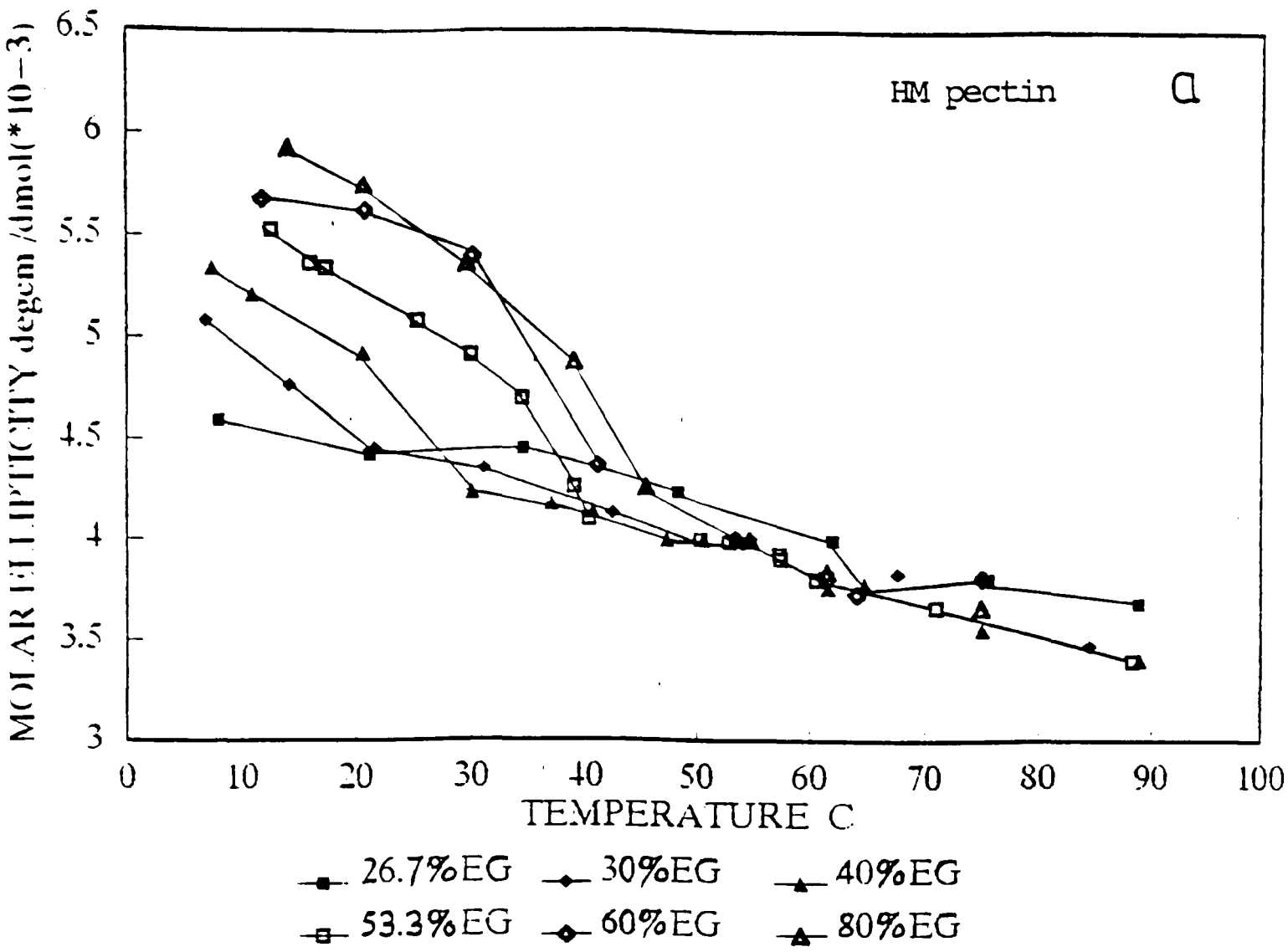


Figure 4.2 CD changes for the first cooling of both a) high methoxy and b) very high methoxy pectin, 1mm path length, 1% concentration.

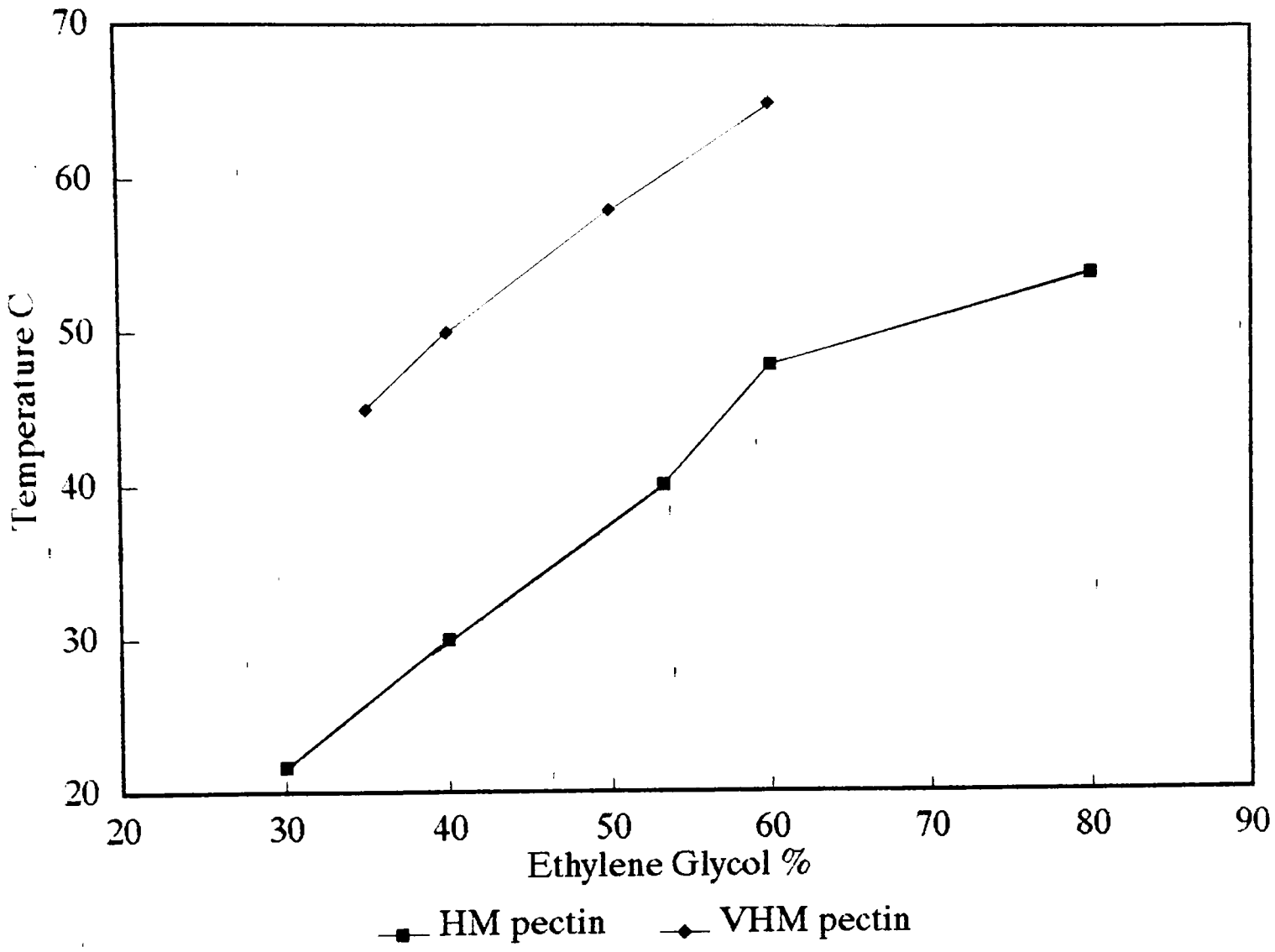


Figure 4.3 Onset of ordering measured at the point of increase in CD for both high methoxy and very high methoxy pectin.

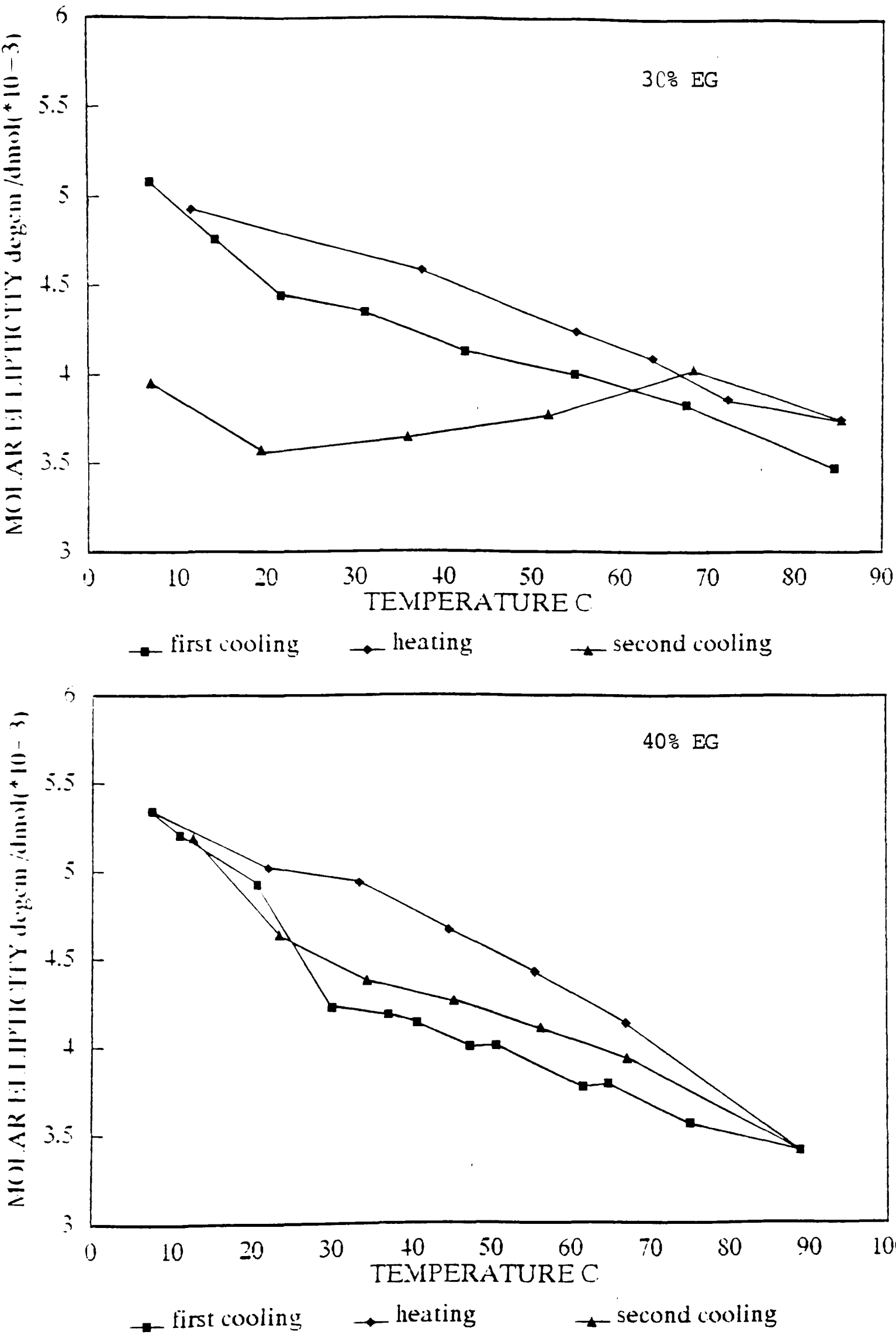
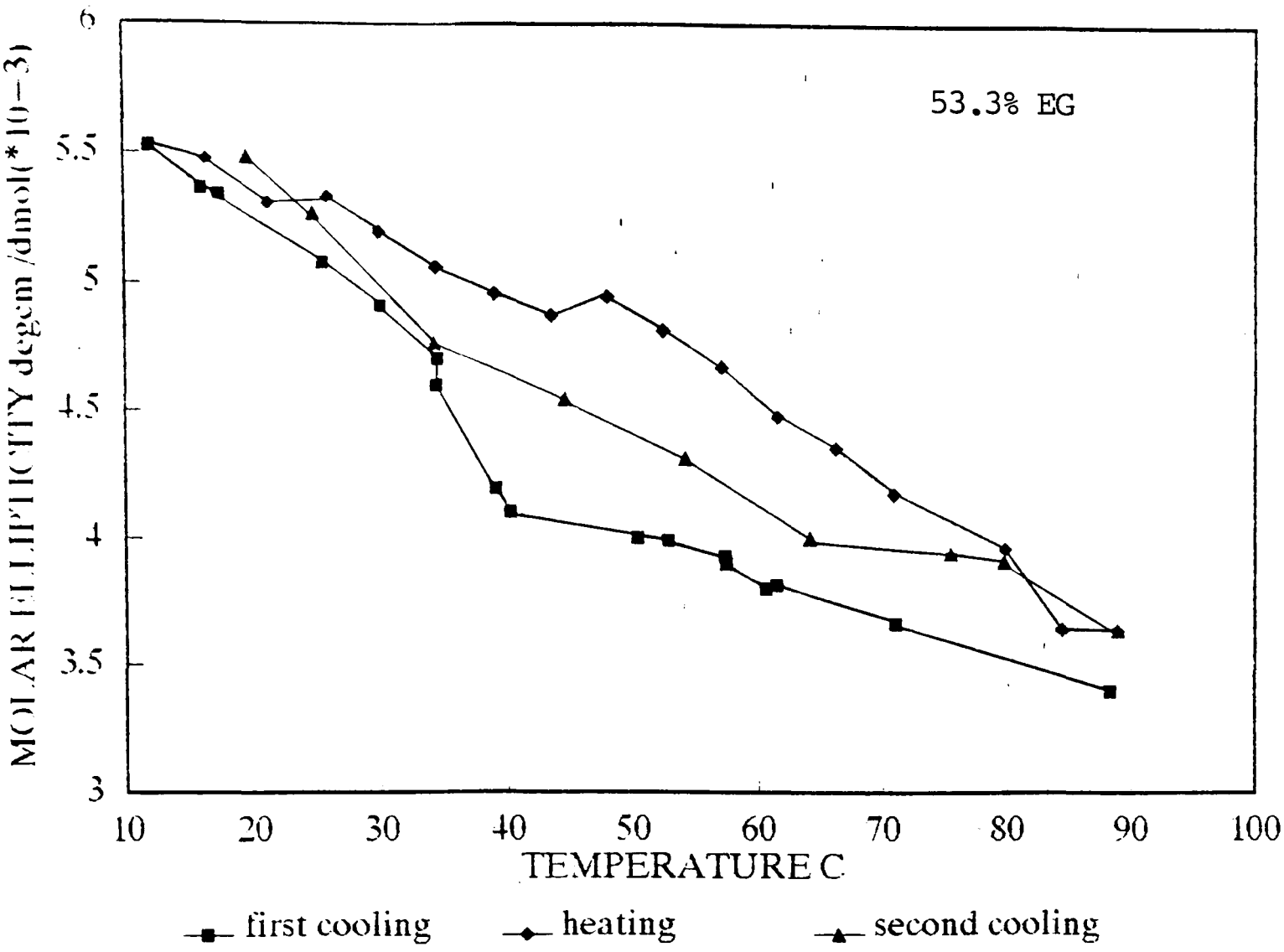
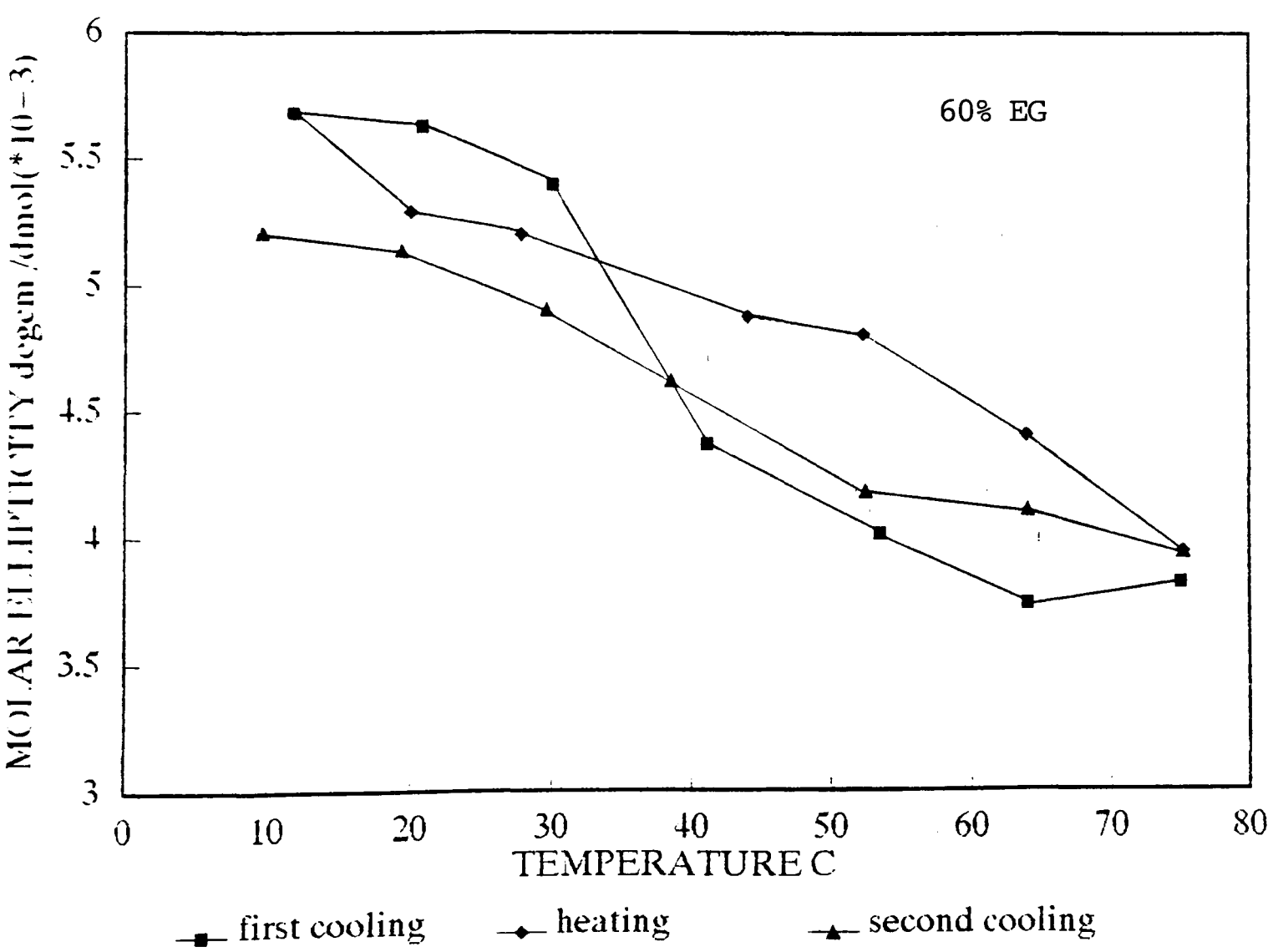
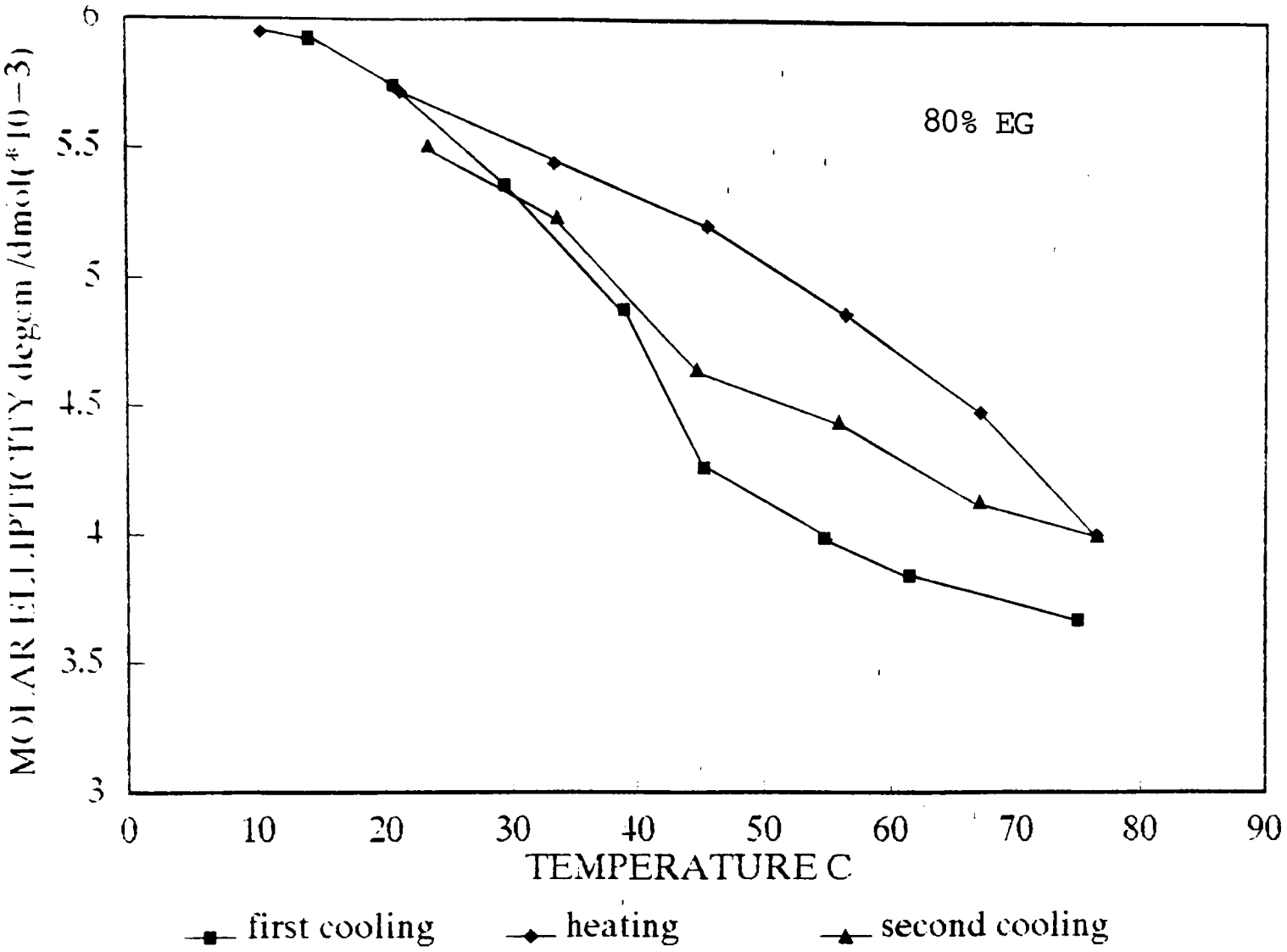


Figure 4.4 Changes in CD on first cooling, heating and second cooling of high methoxy pectin with varying concentrations of ethylene glycol.


53.3% EG
MOLAR ELLIPTICITY degcm /dmol(*10−3)
6
5.5
5
4.5
4
3.5
3
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling


60% EG
MOLAR ELLIPTICITY degcm /dmol(*10−3)
6
4.5
4
3.5
3
0
10
20
30
40
50
60
70
80
TEMPERATURE C
first cooling
heating
second cooling


80% EG
MOLAR ELLIPTICITY degcm /dmol(*10−3)
6
5.5
5
4.5
4
3.5
3
0
10
20
30
40
50
60
70
80
90
TEMPERATURE C
first cooling
heating
second cooling

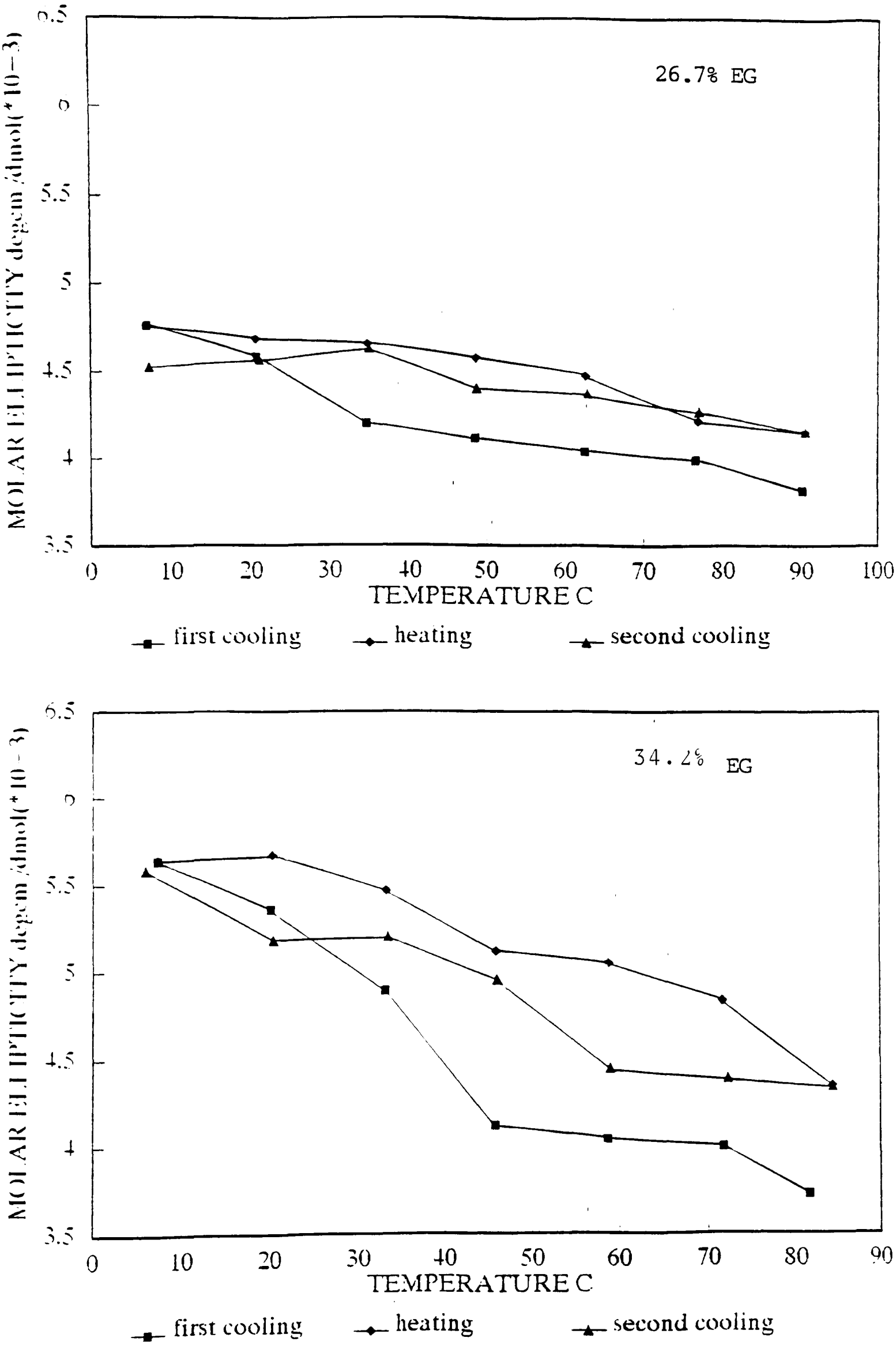
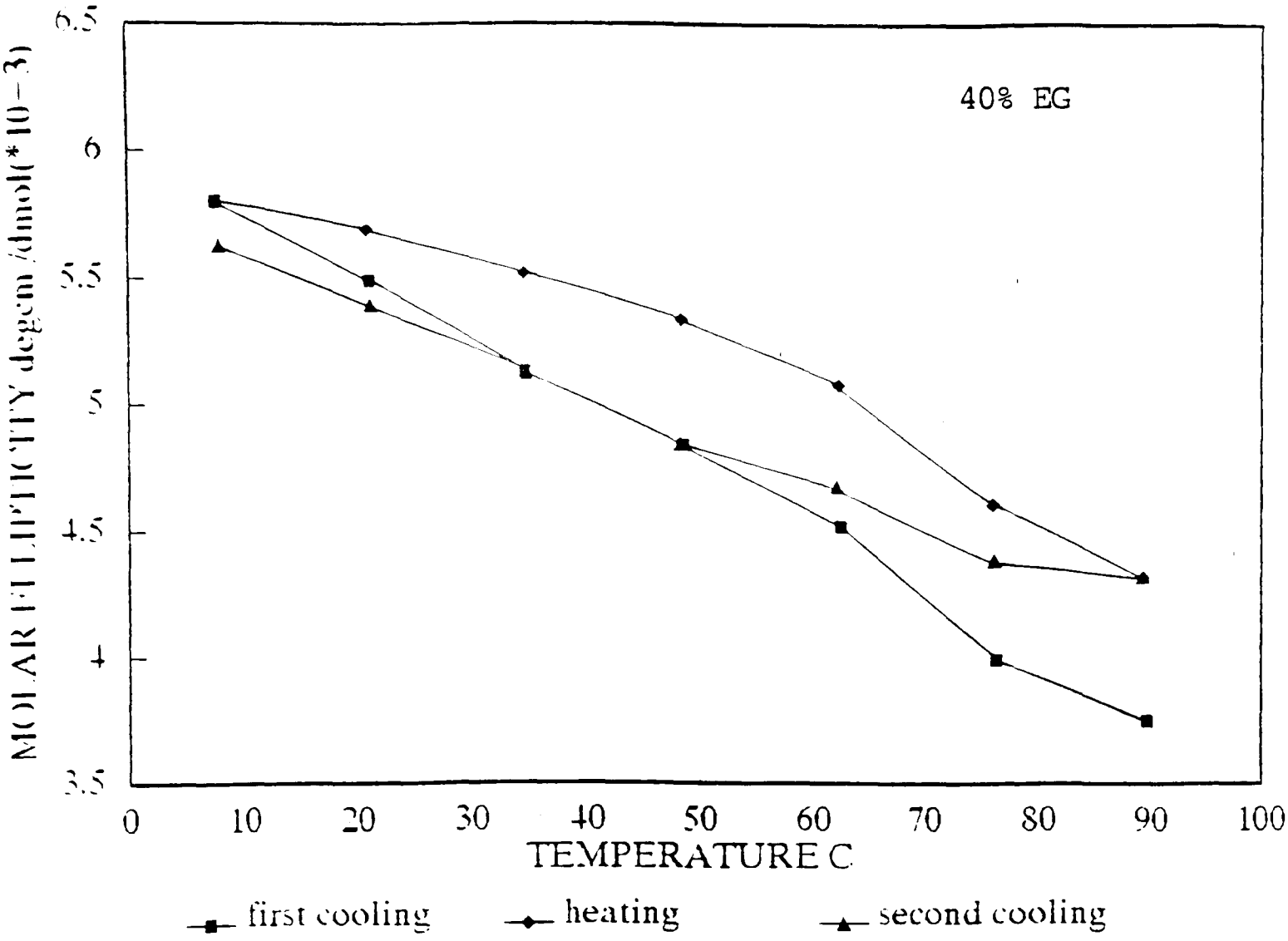
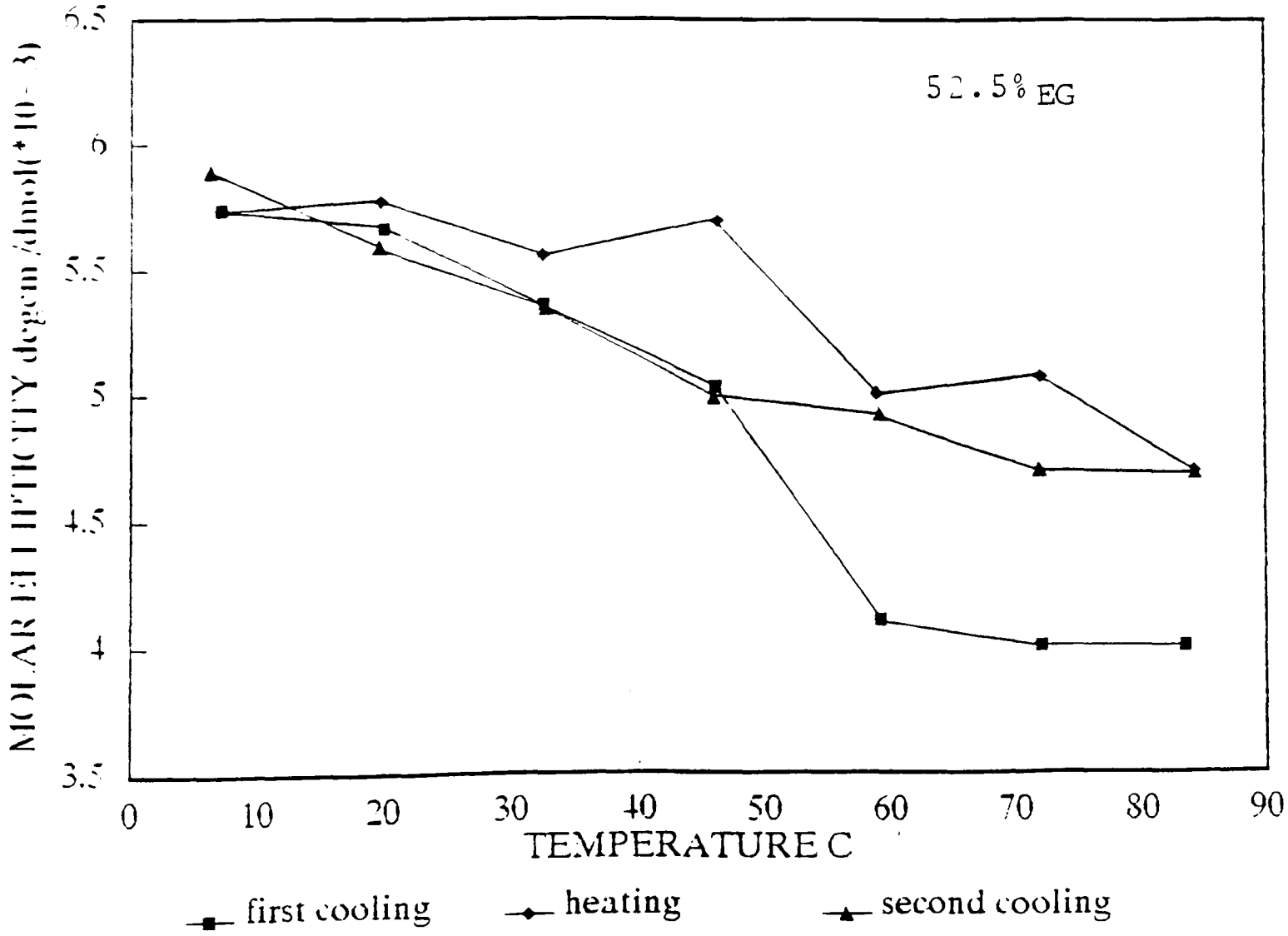
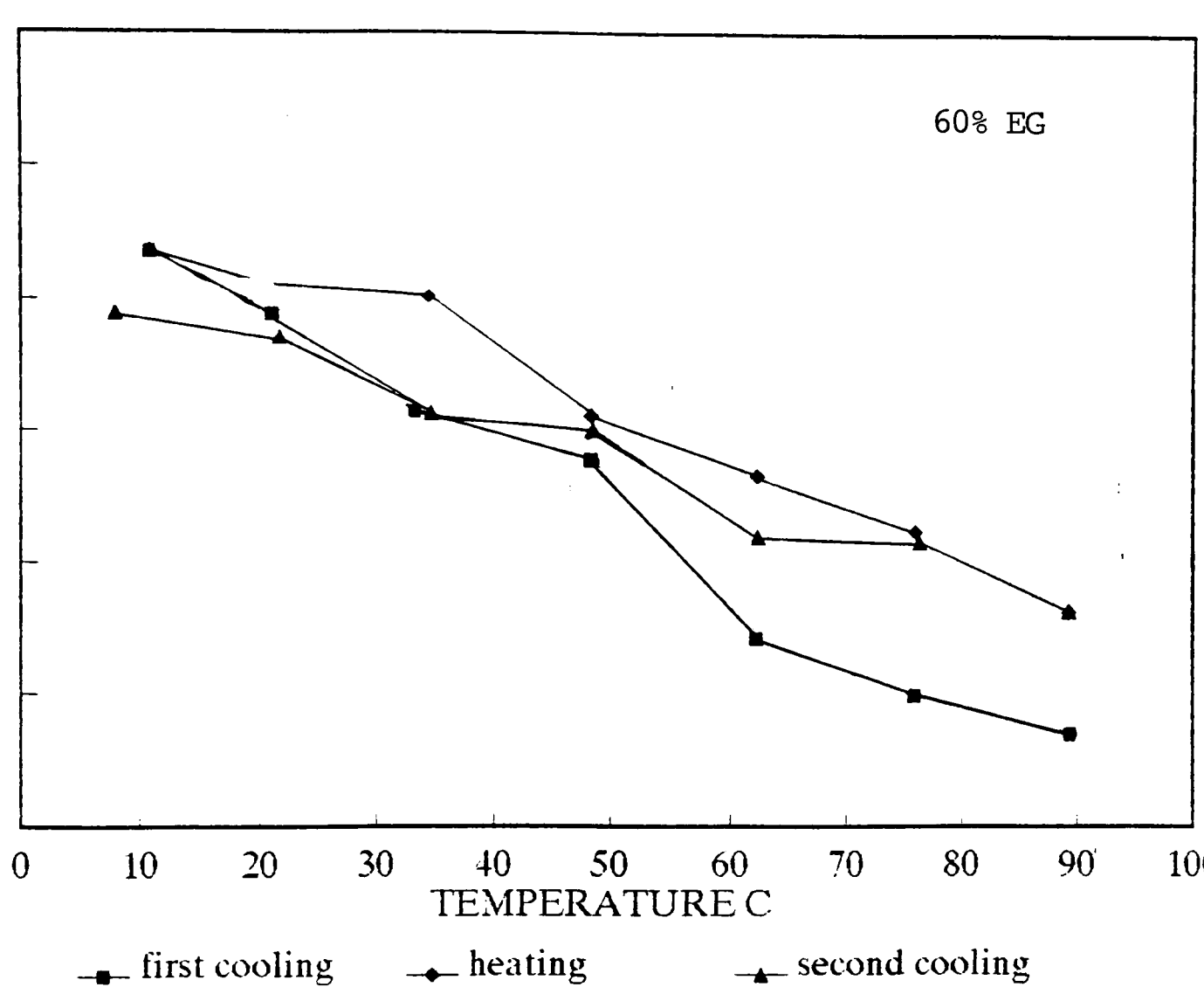


Figure 4.5 Changes in CD on first cooling, heating and second cooling of very high methoxy pectin with varying concentrations of ethylene glycol.


40% EG
6.5
6
5.5
5
4.5
4
3.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling


52.5% EG
6
5
4
0
10
20
30
40
50
60
70
80
90
TEMPERATURE C
first cooling
heating
second cooling


60% EG
MOLAR ELLIPTICITY degcm /dmol(*10−3)
6.5
6
5.5
5
4.5
4
3.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling

4.3 Optical Rotation

Although the broad conclusions from the CD study reported in the previous section seem reasonably clear, and are consistent with previous evidence (Morris *et al.*, 1980), the absolute changes in CD (Figure 4.1) are comparatively small, leading to the large degree of scatter of experimental results evident in Figures 4.4 and 4.5. The related chiroptical technique of optical rotation has therefore been used, in an attempt to define more precisely the temperature course of conformational change.

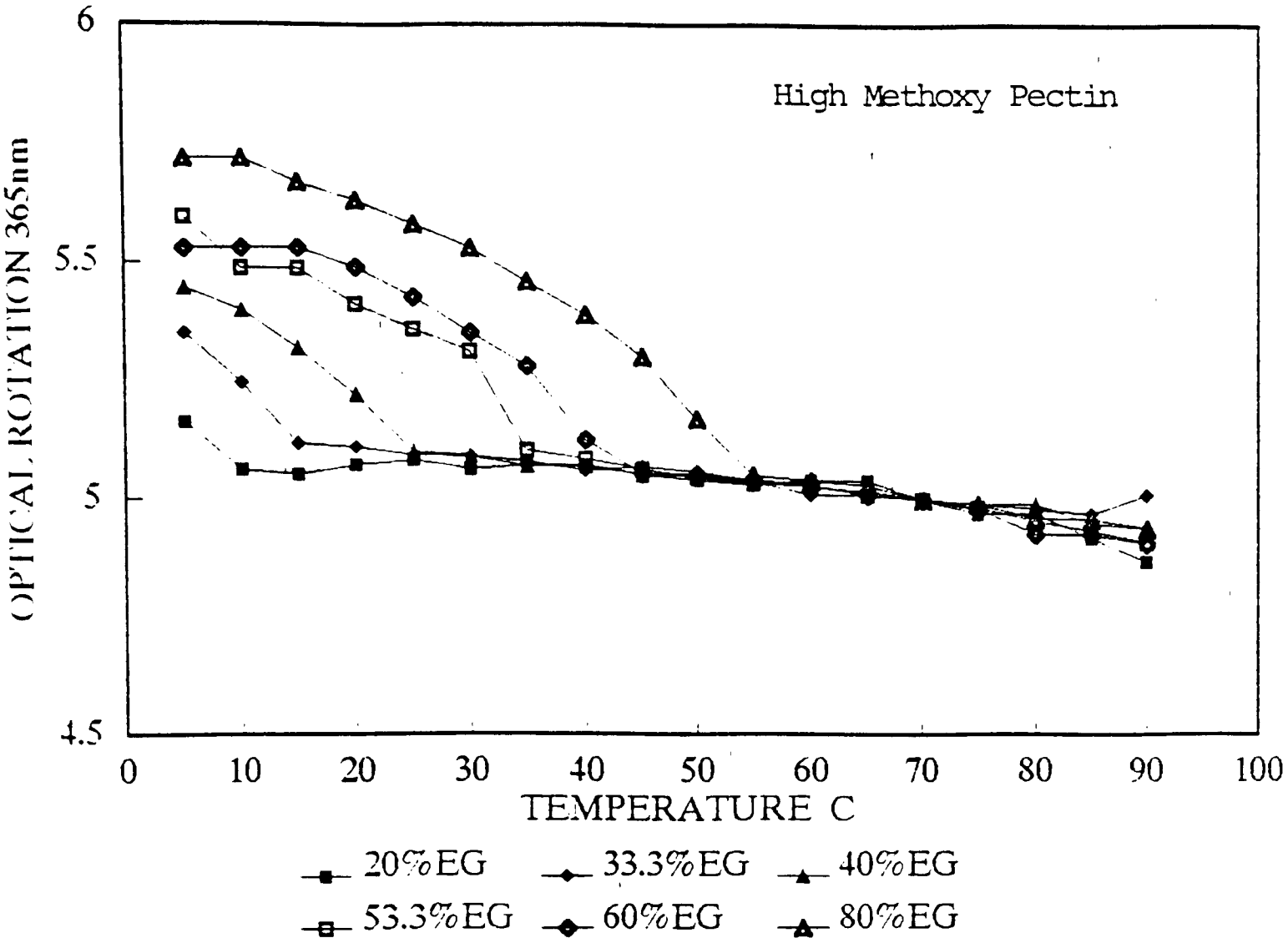
The samples were prepared from a stock solution (Section 3.1) and maintained at high temperatures prior to loading into the heated OR cells. First cooling from 95 to 5°C was followed by a heating regime to 95°C and a second cooling back to 5°C.

Figure 4.6 shows the changes in OR over the temperature range of the sol-gel transitions for both pectin samples. As reported for CD, the transition moves to higher temperatures with increasing ethylene glycol concentration, and is consistently higher, by about 20°C, for very high methoxy pectin than high methoxy pectin (Figure 4.7). The transition is fairly co-operative for both series of pectins, depicted by a very clear, relatively sharp increase in OR.

As seen for CD, the conformational change on cooling is extensively irreversible with only a slight loss of OR at high temperatures restored by subsequent re-cooling but running substantially below the heating curve (Figures 4.8 and 4.9). Again the decrease on heating is much less for very high methoxy pectin than high methoxy pectin and the hysteresis is enhanced.

It would appear from these results (Section 4.2 and 4.3), indicating a higher temperature for the onset of the conformational transition, that very high methoxy pectin is capable of adopting the ordered structure more easily than high methoxy pectin despite losing the ability

to form a cohesive gel network in the presence of ethylene glycol. The mechanism for gelation proposed from this evidence is presented in Section 4.6.



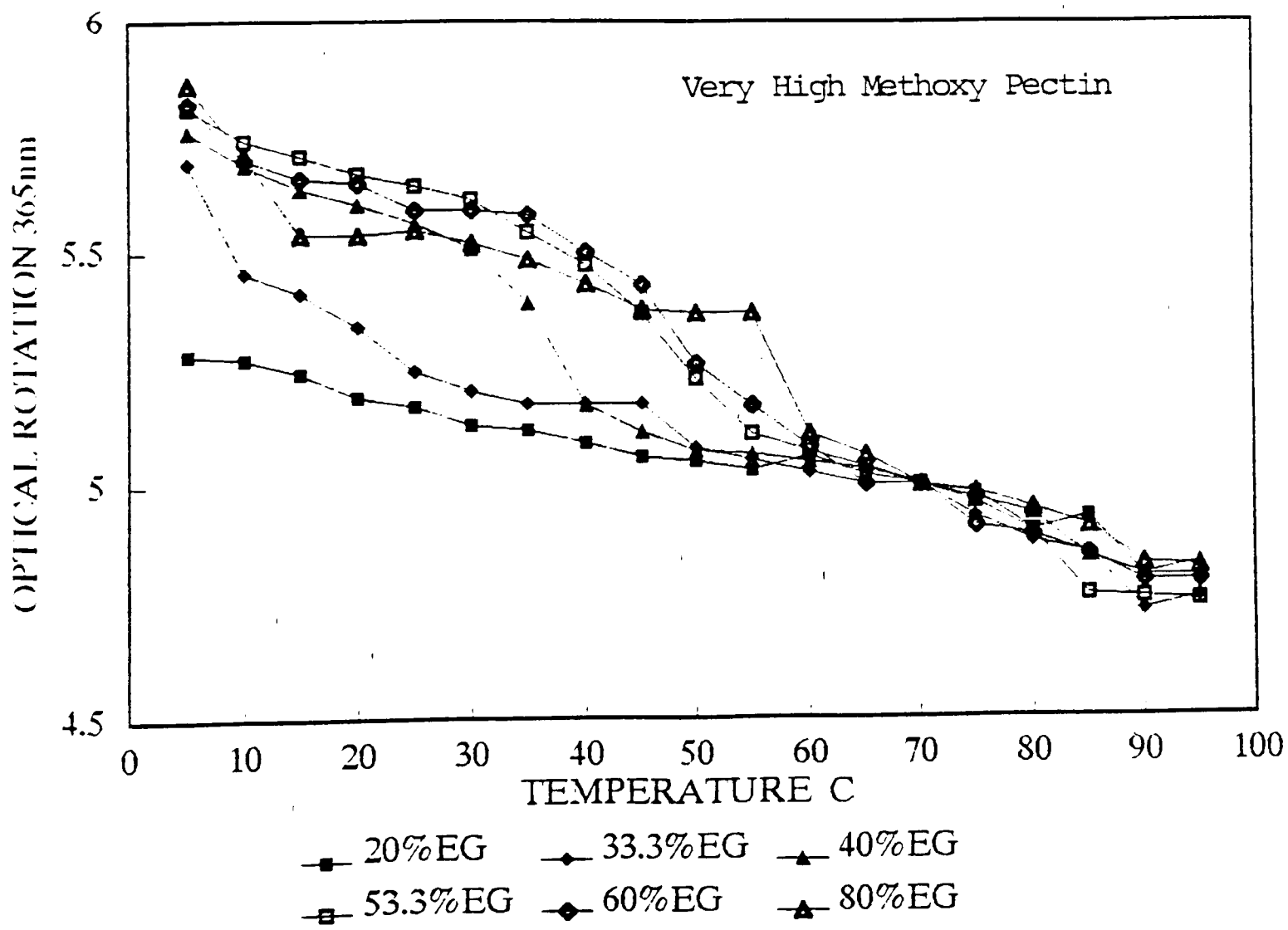


Figure 4.6 Changes in OR on first cooling, of high methoxy pectin with varying concentrations of ethylene glycol.

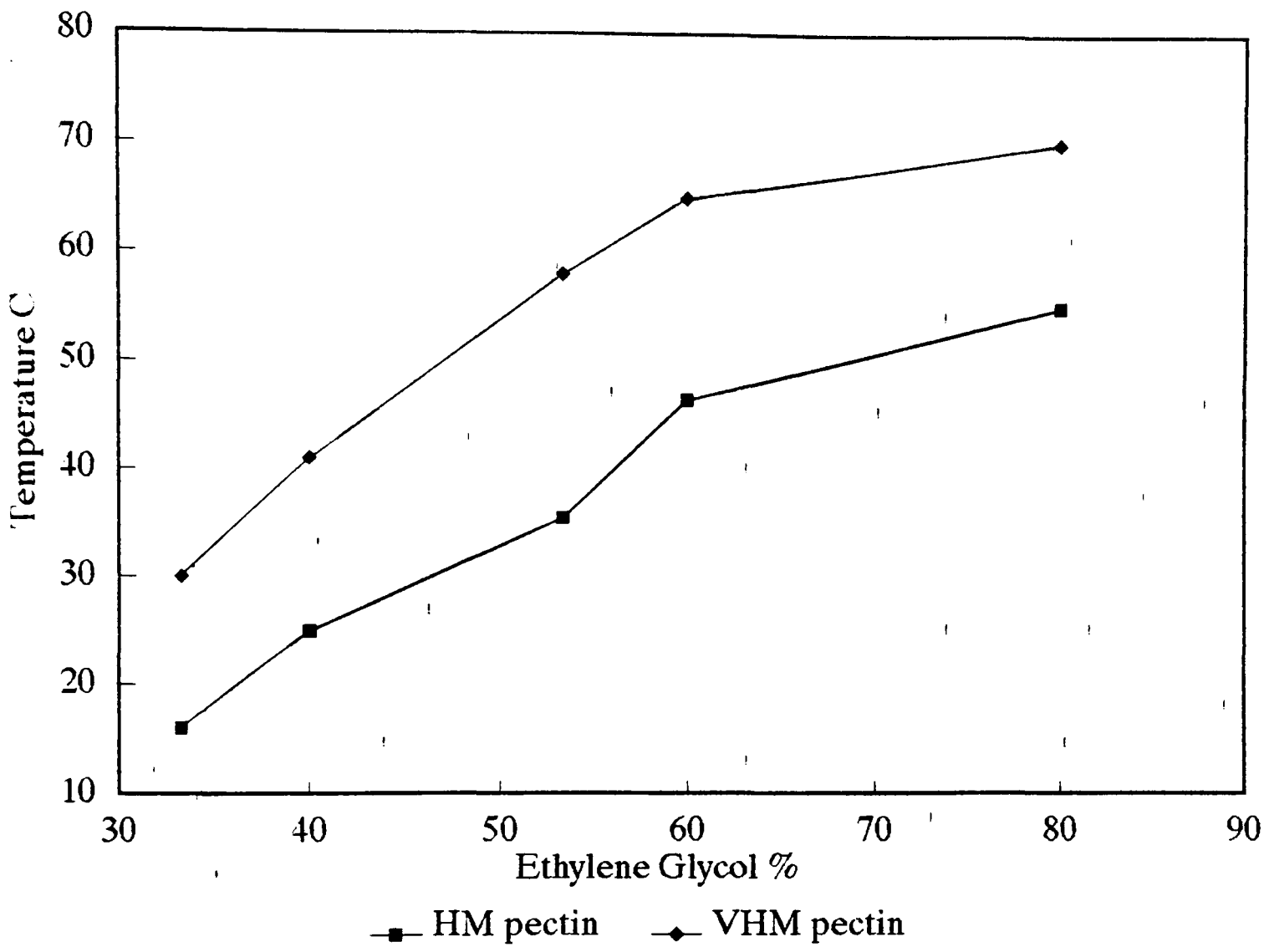
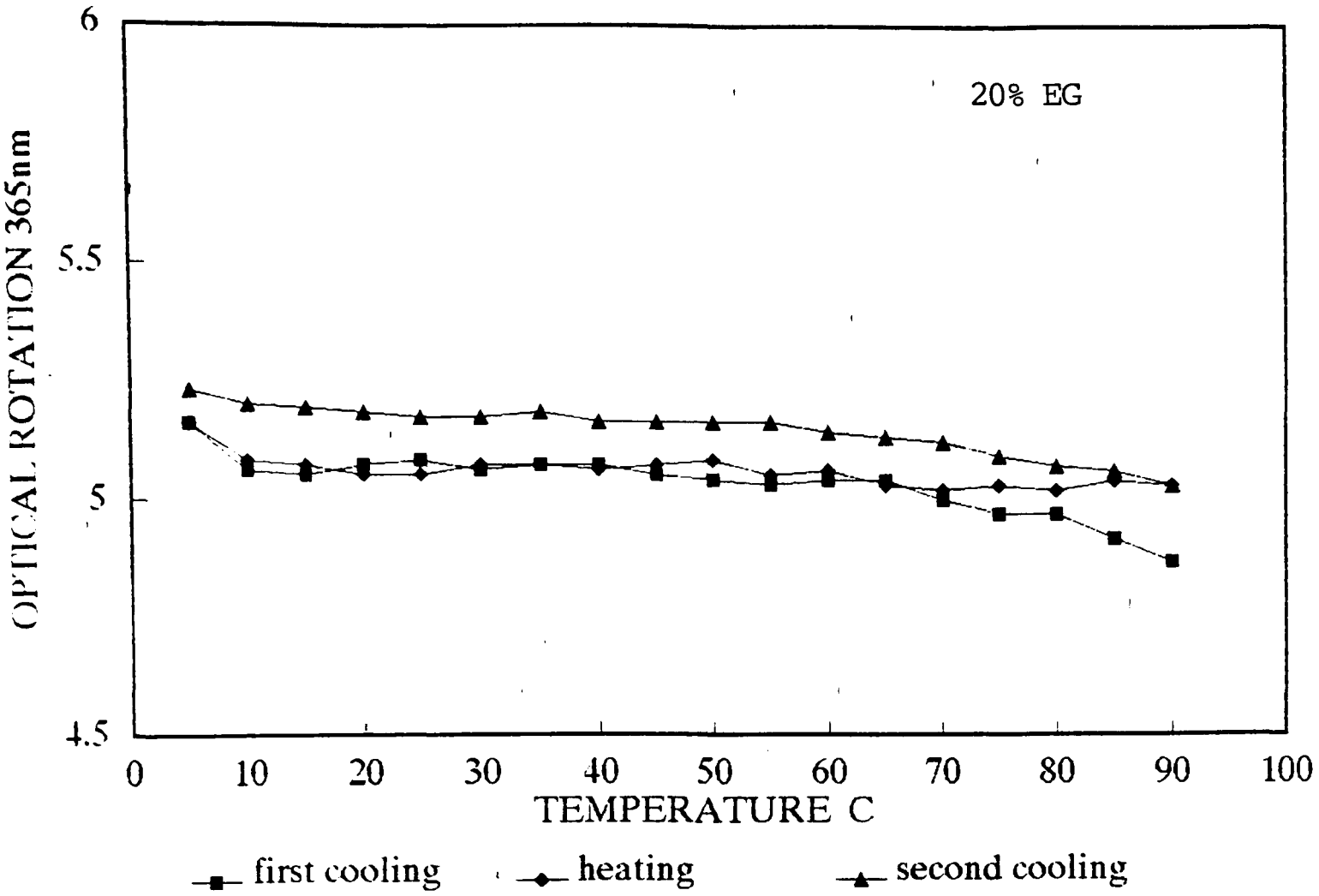


Figure 4.7 Onset of ordering measured at the point of increase in OR for both high methoxy and very high methoxy pectin.



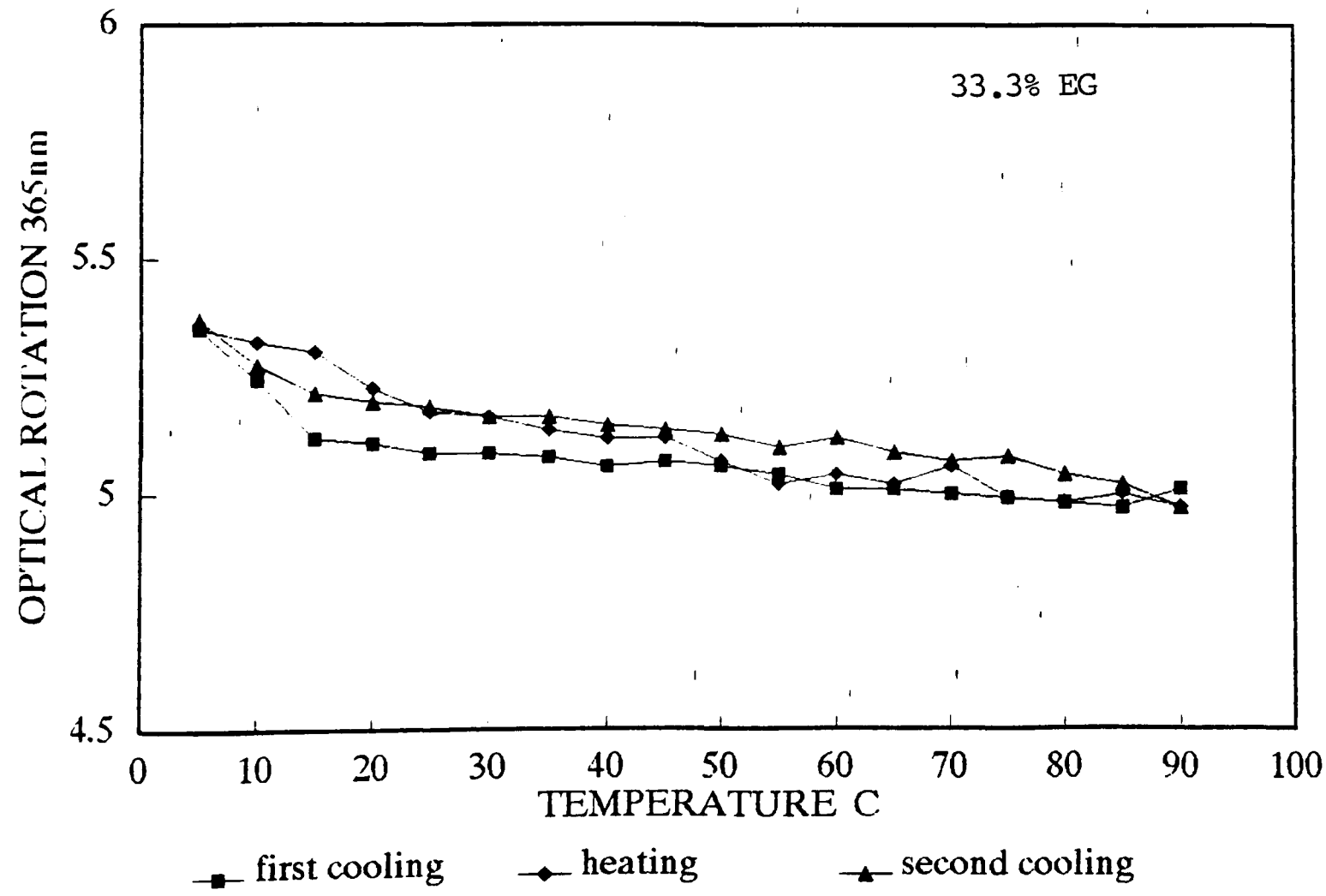
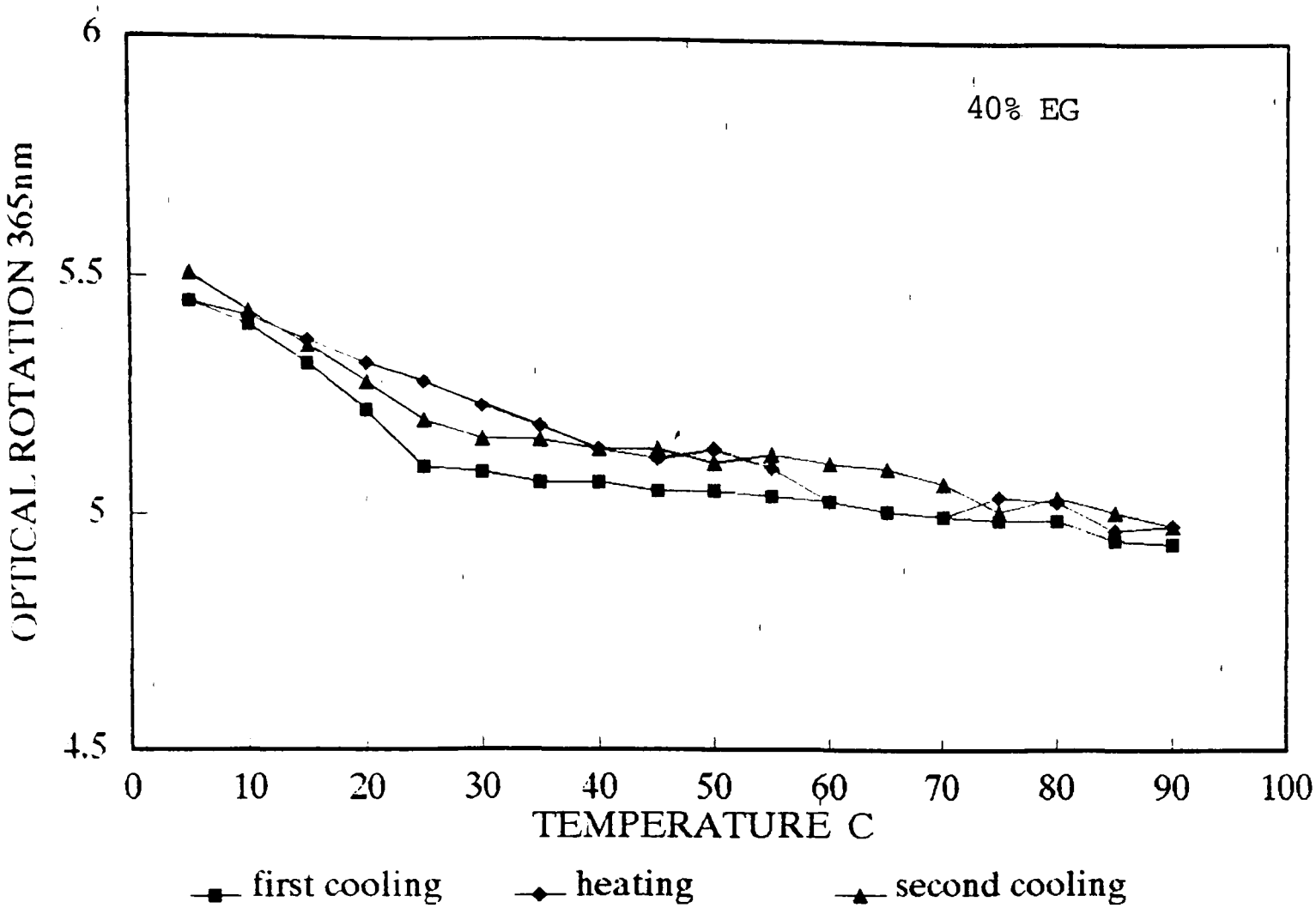
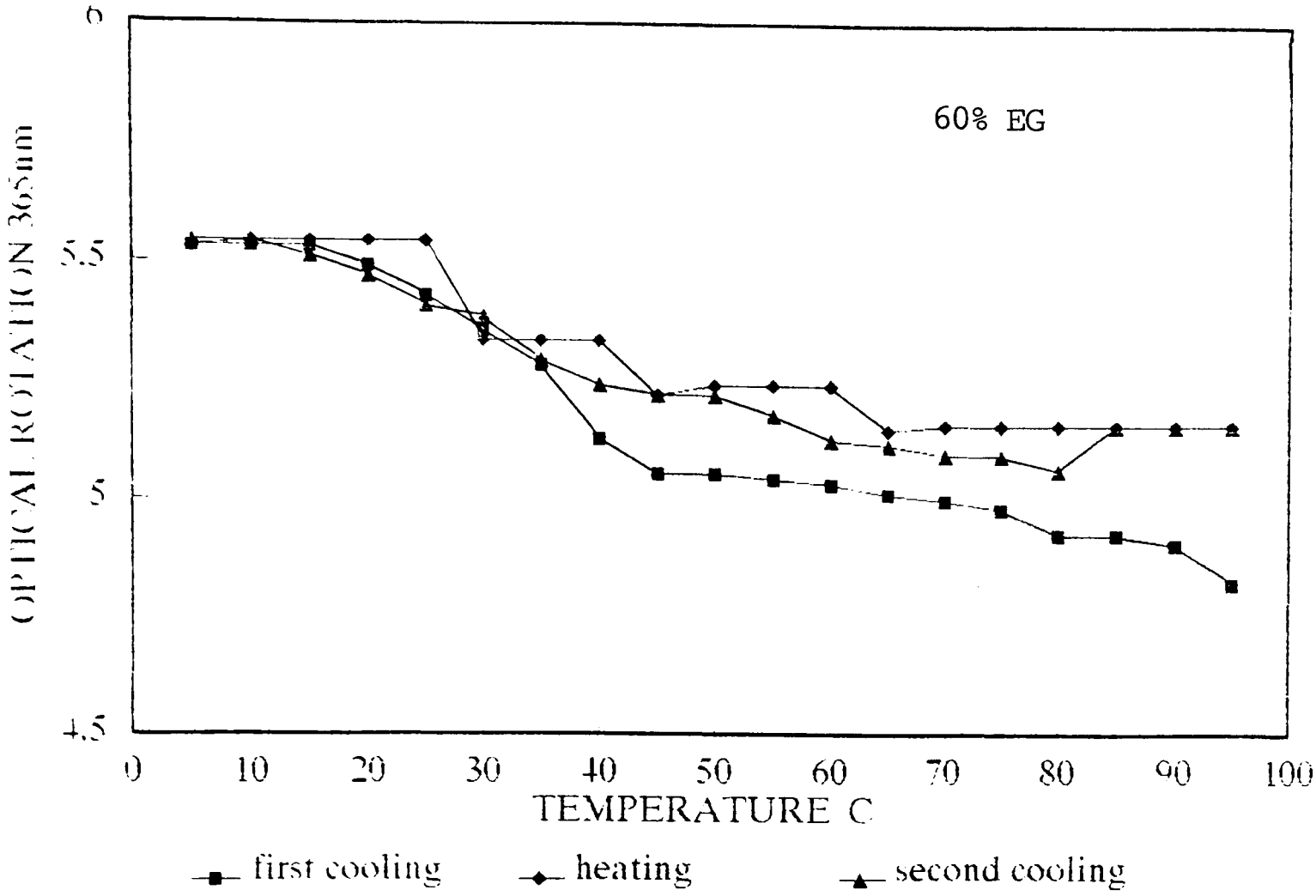
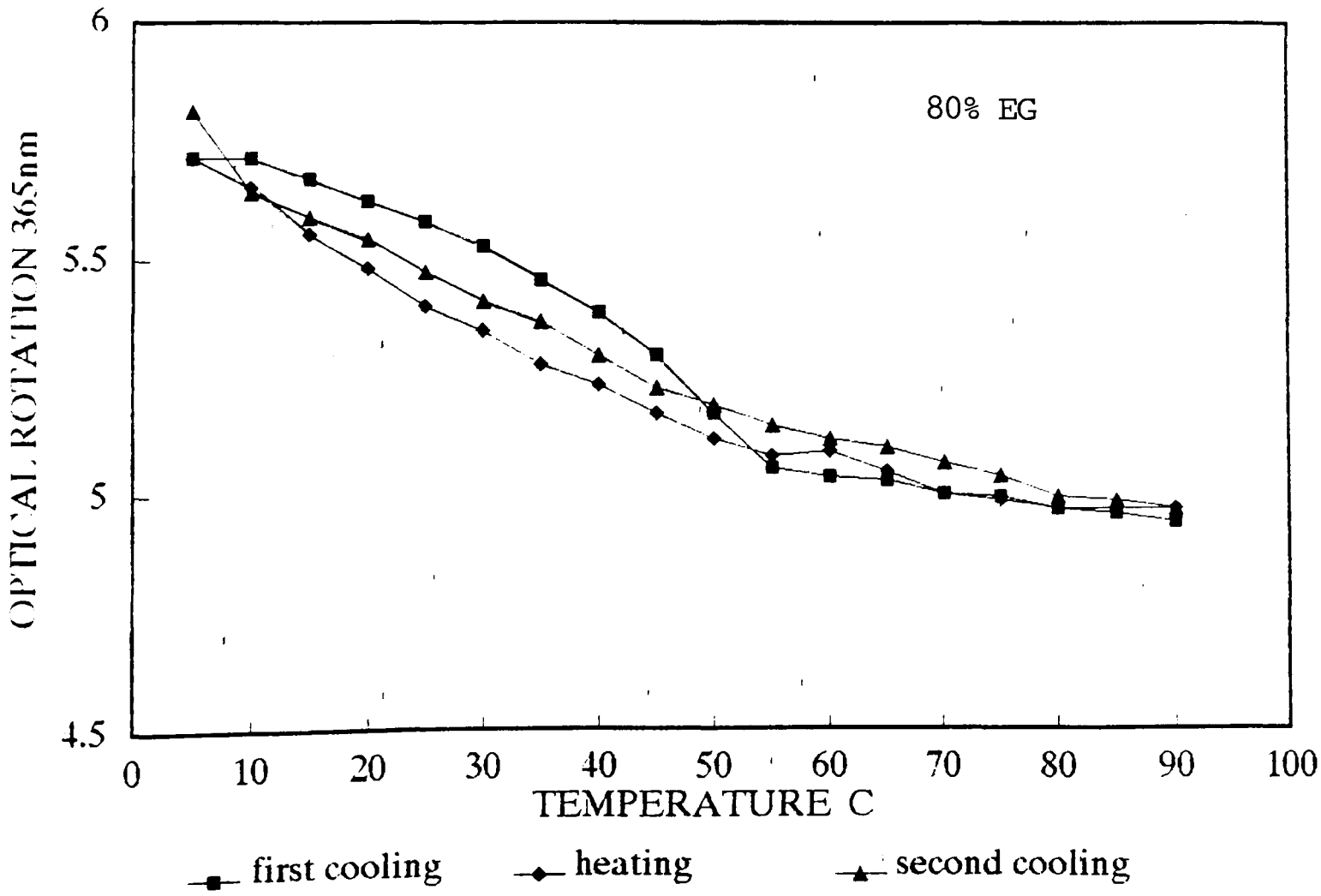


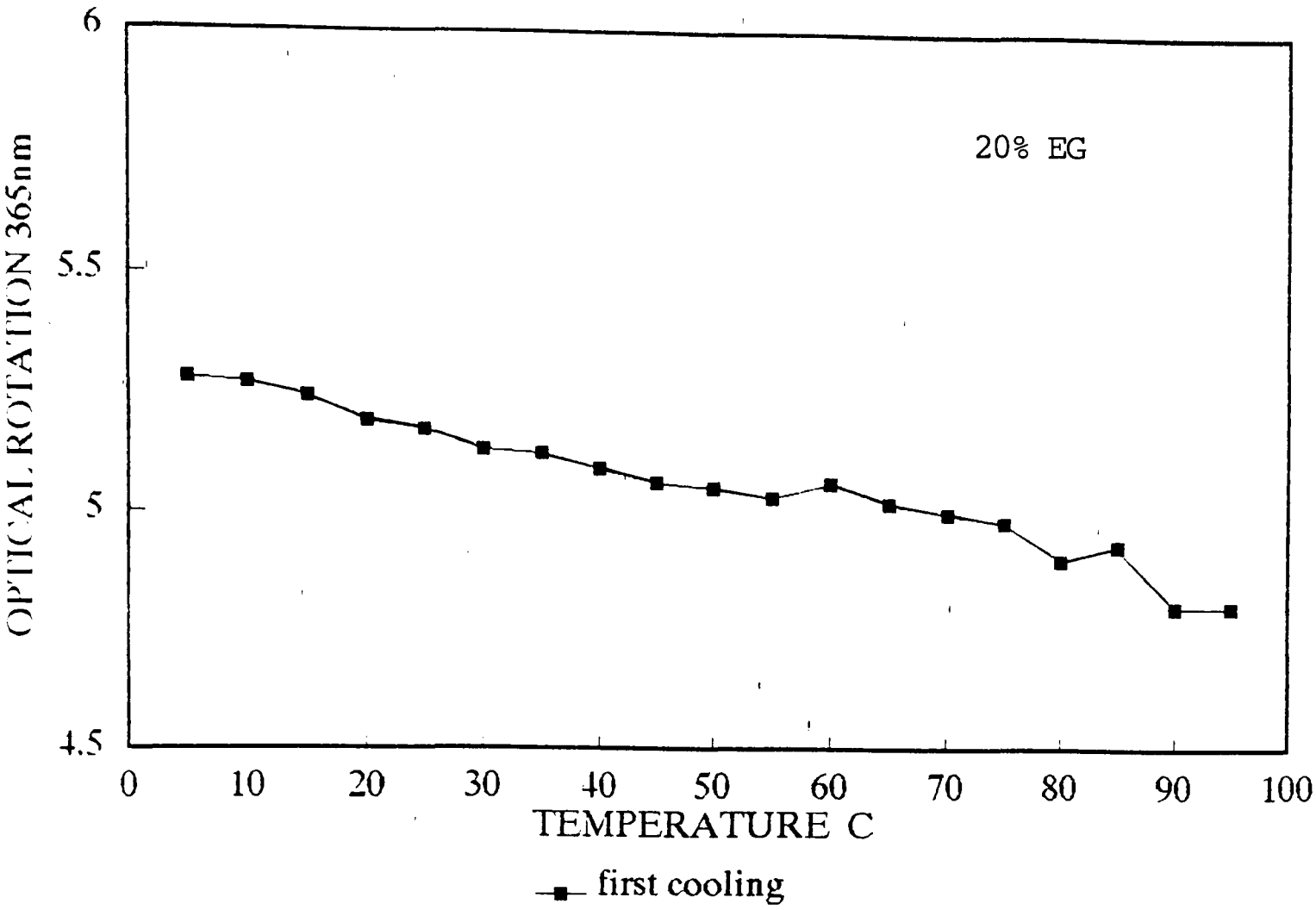
Figure 4.8 Changes in OR on first cooling, heating and second cooling of high methoxy pectin with varying concentrations of ethylene glycol.


40% EG
OPTICAL ROTATION 365nm
6
5.5
5
4.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling

53.3% EG
OPTICAL ROTATION 365nm
6
5.5
5
4.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling


60% EG
OPTICAL ROTATION 365nm
6
5.5
5
4.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling


80% EG
OPTICAL ROTATION 365nm
6
5.5
5
4.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling



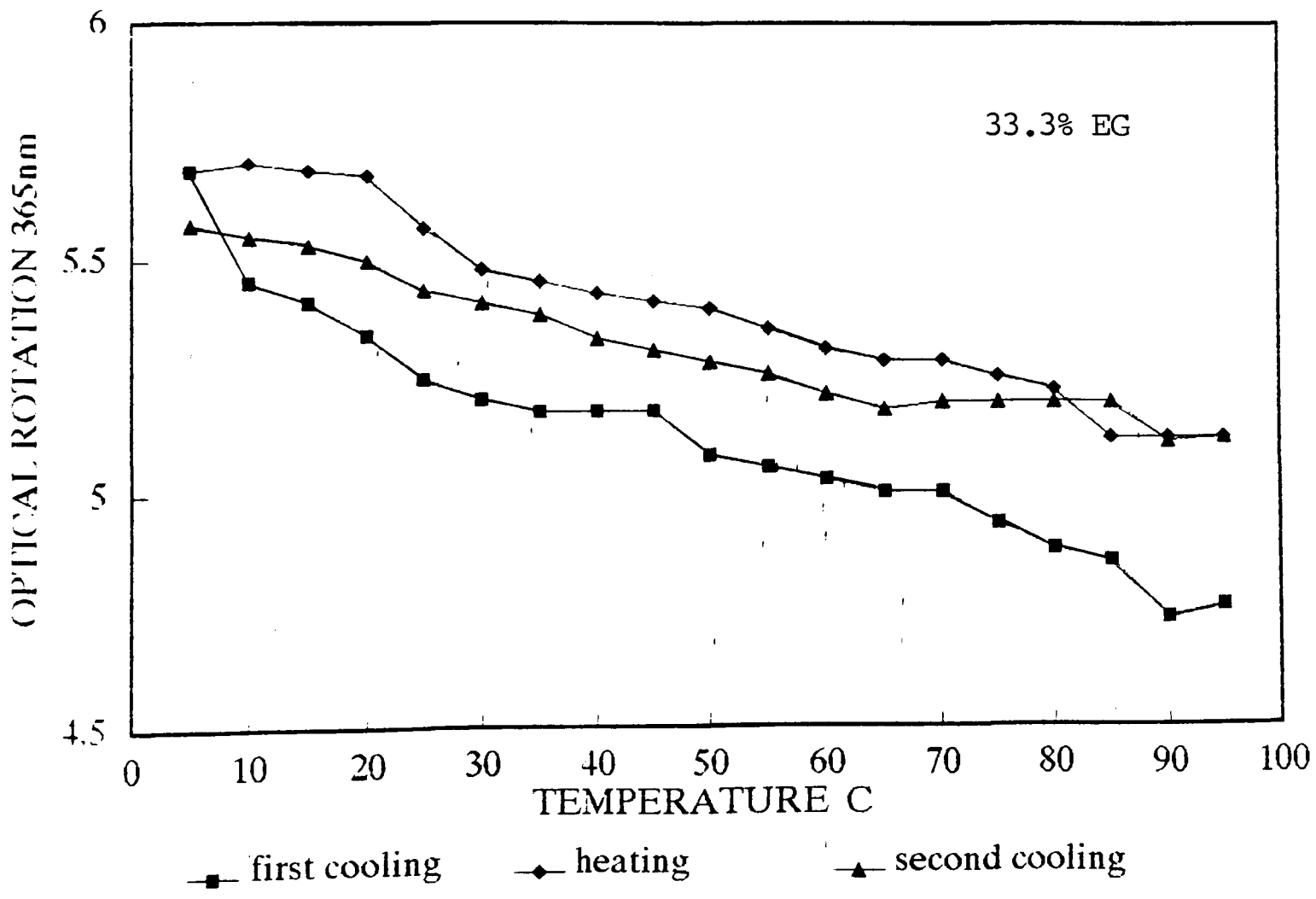
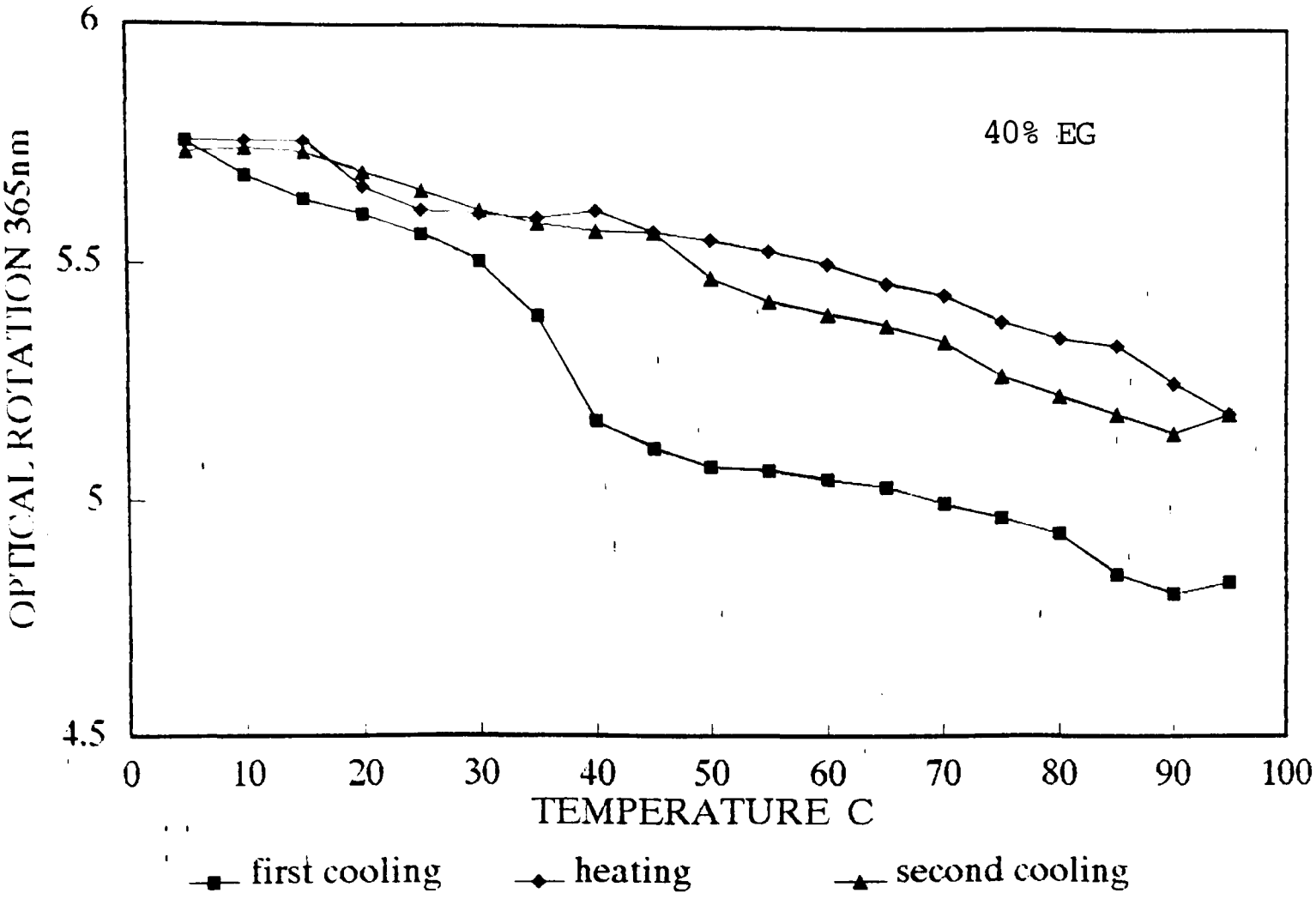
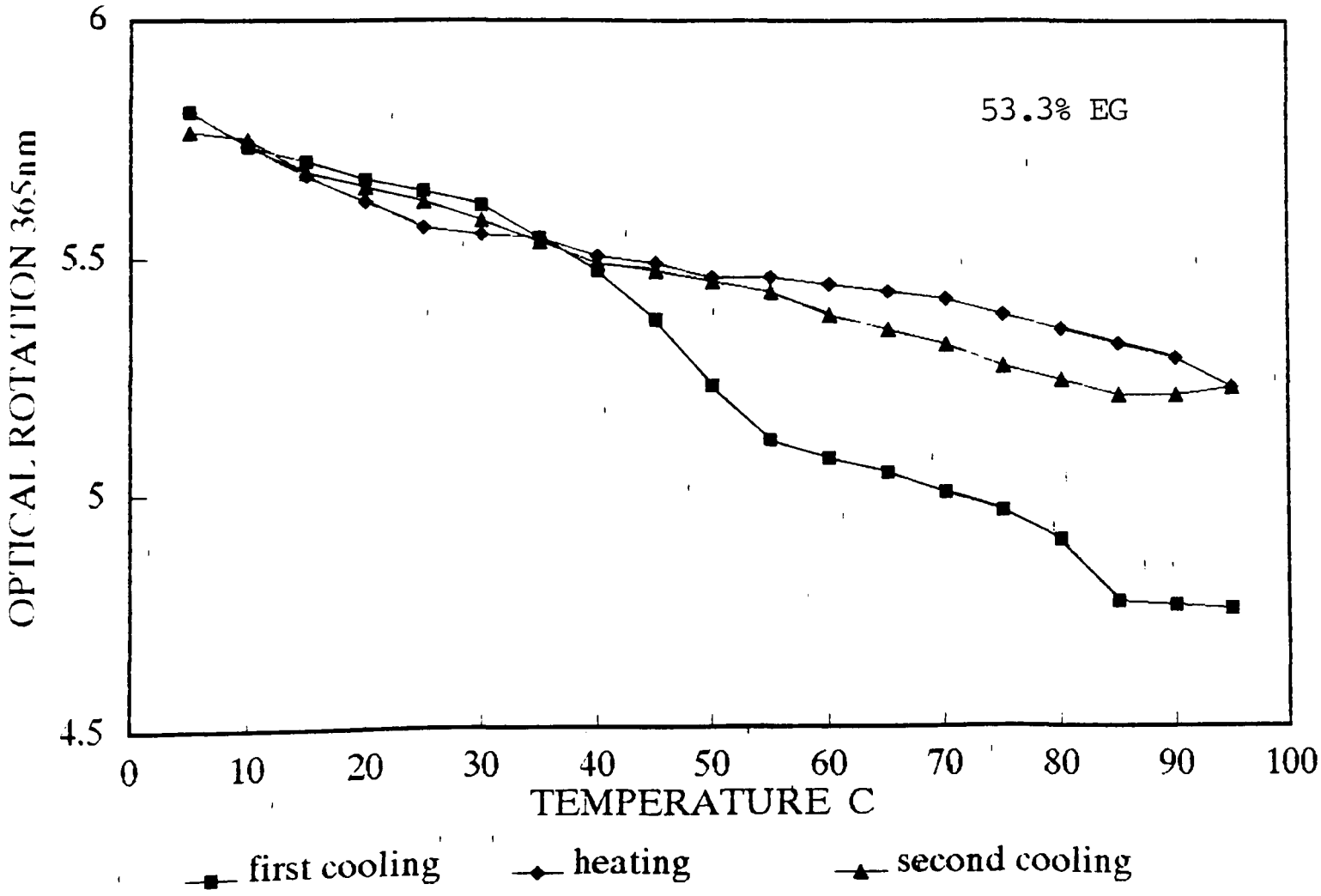
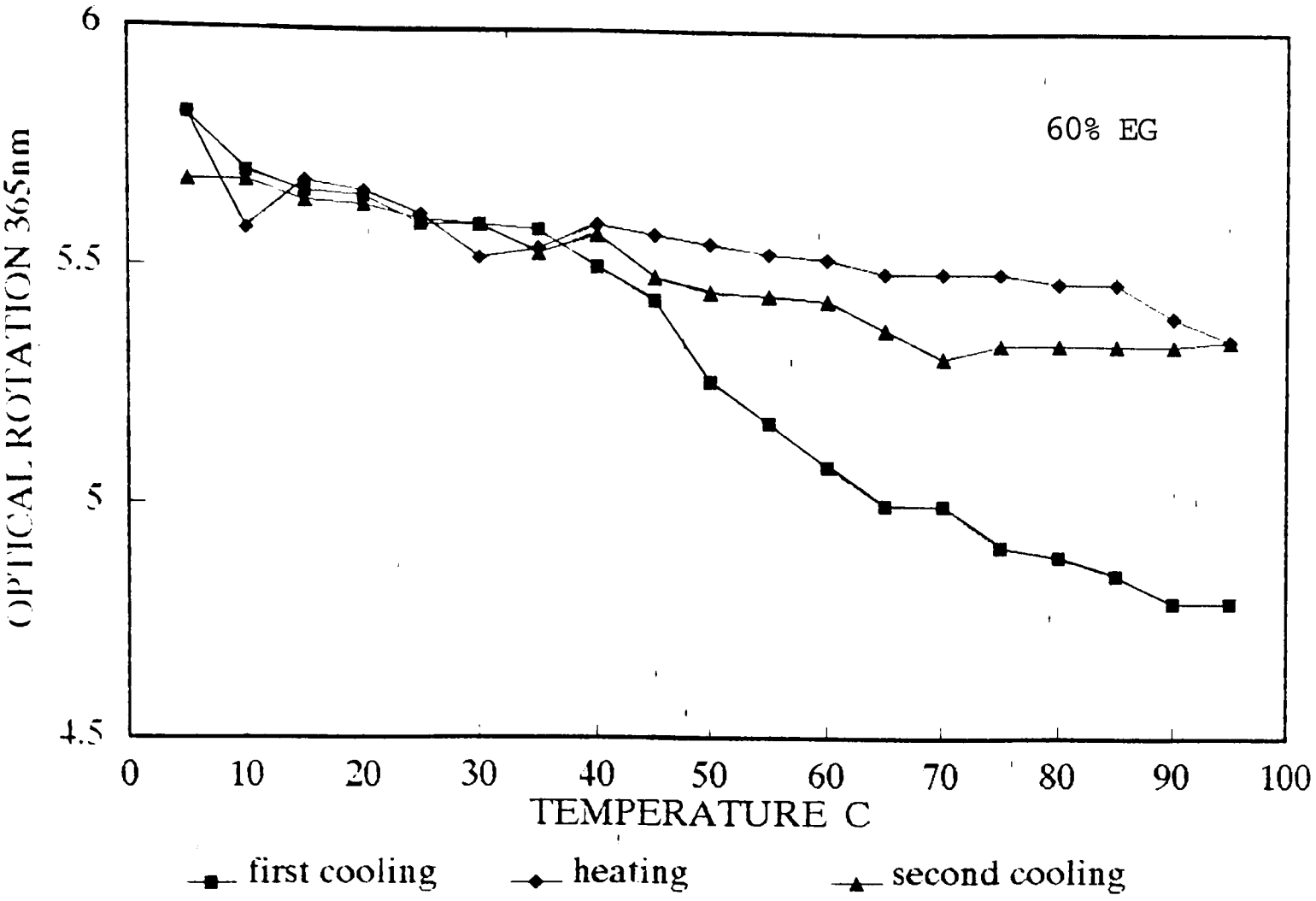
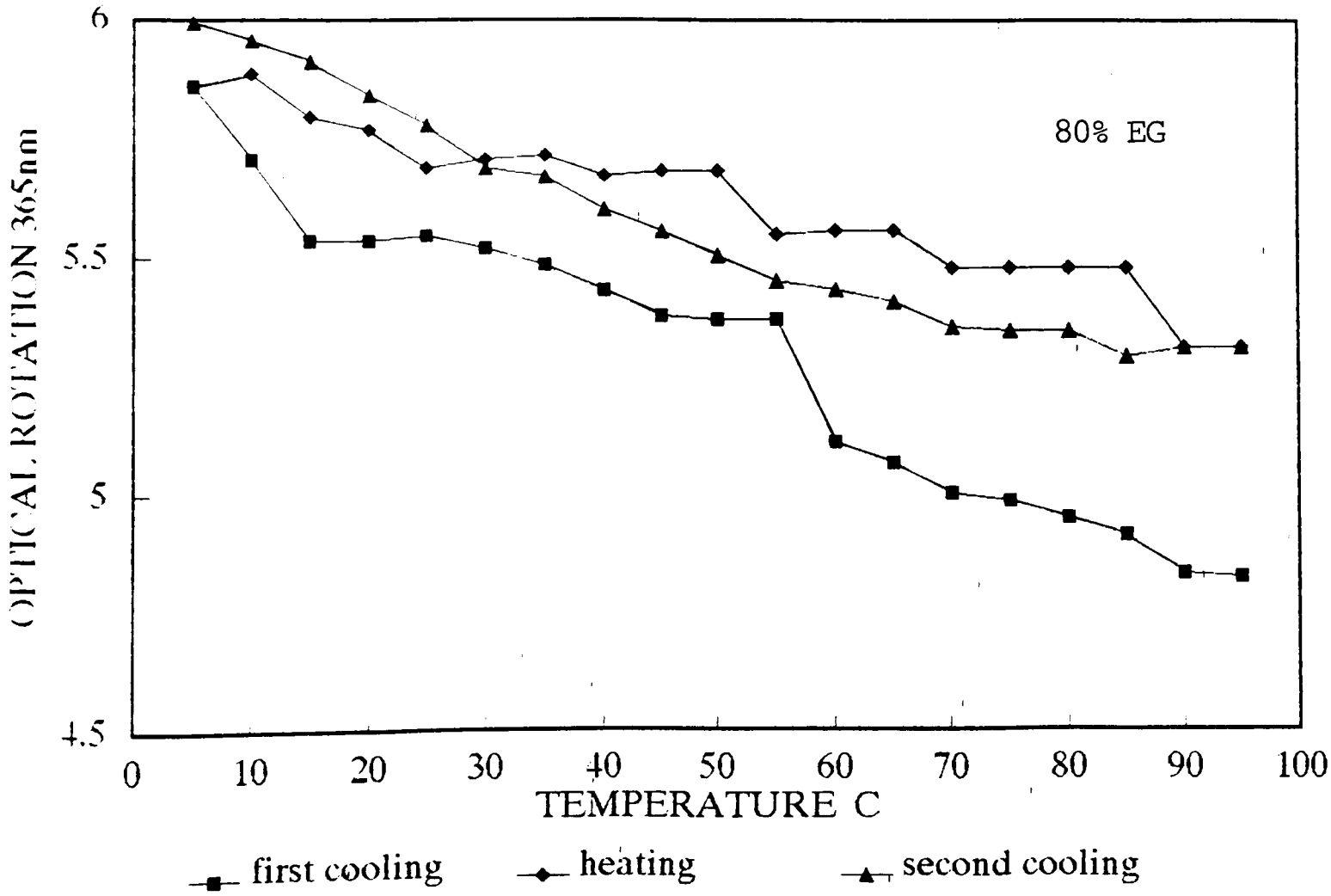


Figure 4.9 Changes in OR on first cooling, heating and second cooling of very high methoxy pectin with varying concentrations of ethylene glycol.


40% EG
OPTICAL ROTATION 365nm
6
5.5
5
4.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling


53.3% EG
OPTICAL ROTATION 365nm
6
5.5
5
4.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling


60% EG
OPTICAL ROTATION 365nm
6
5.5
5
4.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling


80% EG
OPTICAL ROTATION 365nm
6
5.5
5
4.5
0
10
20
30
40
50
60
70
80
90
100
TEMPERATURE C
first cooling
heating
second cooling

4.4 Mechanical Spectroscopy

Chiroptical techniques have been used to demonstrate the formation of a conformationally ordered structure in high and very high methoxy pectin by following the transition occurring with decreasing temperature. Mechanical spectroscopy is employed here to follow the structural progression of gelation occurring in the presence of the solvent modifier; ethylene glycol.

The samples were prepared in exactly the same way as for chiroptical measurements in Section 4.2 and 4.3, and maintained at high temperature prior to loading onto the pre-heated plate of the rheometer. The sample was subjected to an oscillation of set frequency and strain whilst cooling at a rate of 1 degree per minute from 90°C to 5°C. This was followed by a heating regime to 90°C and a second cooling back to 5°C. Readings for the rigidity modulus (G'), viscous modulus (G") and dynamic viscosity (η^*) variation with frequency were obtained, and a measure of the 'phase lag', δ, giving an indication of the relative liquid-like and solid-like character of the sample.

Gelation is characterised by a sharp increase in 'solid-like' character of the sample (storage modulus, G'; section 2.6) on cooling. Figures 4.10 to 4.19 show the effect of increasing ethylene glycol concentration. During the first cooling there is a gradual increase in both G' and G" with the curves remaining parallel and G' always above G". It is clearly seen that there is a large degree of thermal hysteresis occurring between gel formation and dissociation in both pectin types (Figures 4.20 and 4.21), with the values for subsequent re-cooling running below those of heating until the temperature range of the initial transition as was seen for both CD and OR.

The temperature for the onset of the sol-gel transition (measured at the point of inflection of the G' curve) increases (Figures 4.22 to 4.27) with increasing concentration of ethylene glycol on first cooling, and the slight loss of gel structure during heating, denoted by a fall in

G', occurs at progressively higher temperatures. The gelation temperature is consistently higher for very high methoxy pectin than high methoxy pectin (even for the limited degree of structural organisation in the non-gelling very high methoxy samples with 60 and 80% ethylene glycol).

Figure 4.28 shows a comparison of the temperature course of G', CD and OR on first cooling for both high methoxy and very high methoxy pectin at 60% ethylene glycol. There is a greater degree of scatter for the very high methoxy sample, but a clearly analogous sol-gel, disorder-order, transition is illustrated by all the techniques. The onset of the conformational change (as measured by CD and OR) aligns closely with the increase in gel character measured by mechanical spectroscopy (Figure 4.29).

The loss of gelation observed visually for very high methoxy pectin with high concentrations of ethylene glycol is substantiated by a weakening of the gel structure measured by G' at 20°C during the first cooling (Figure 4.30).

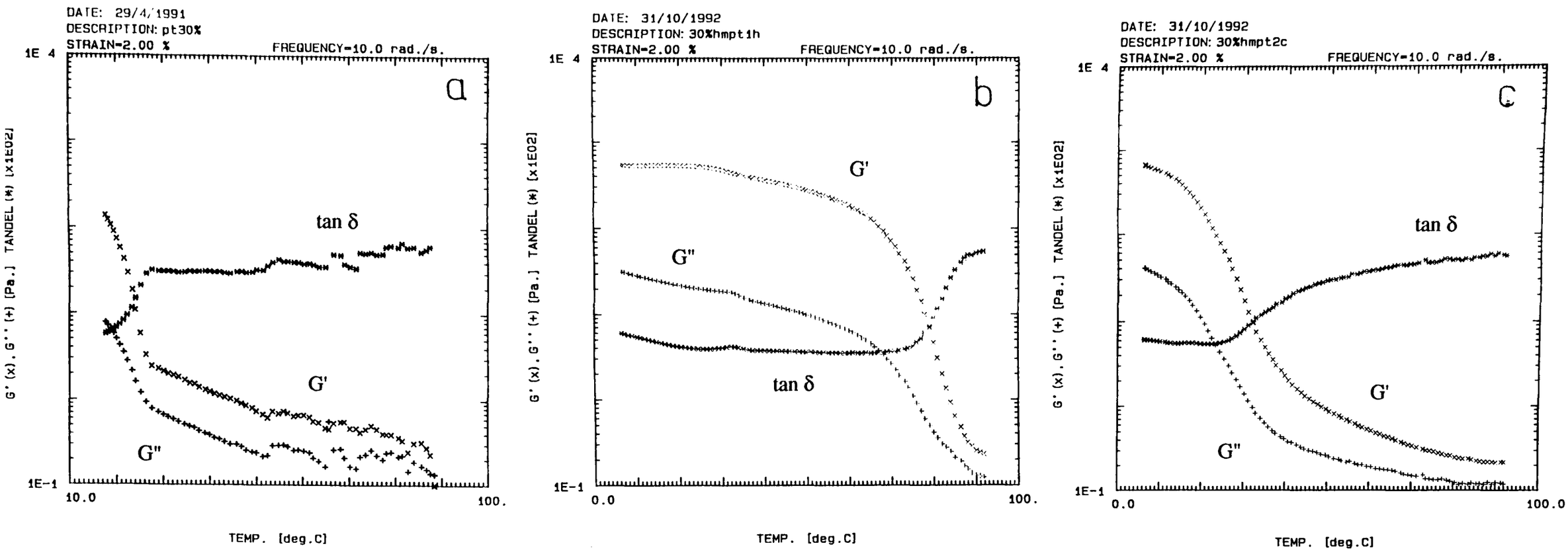


Figure 4.10 Changes in structural moduli of 1% high methoxy pectin with 30% ethylene glycol. a) first cooling, b) heating, c) second cooling.

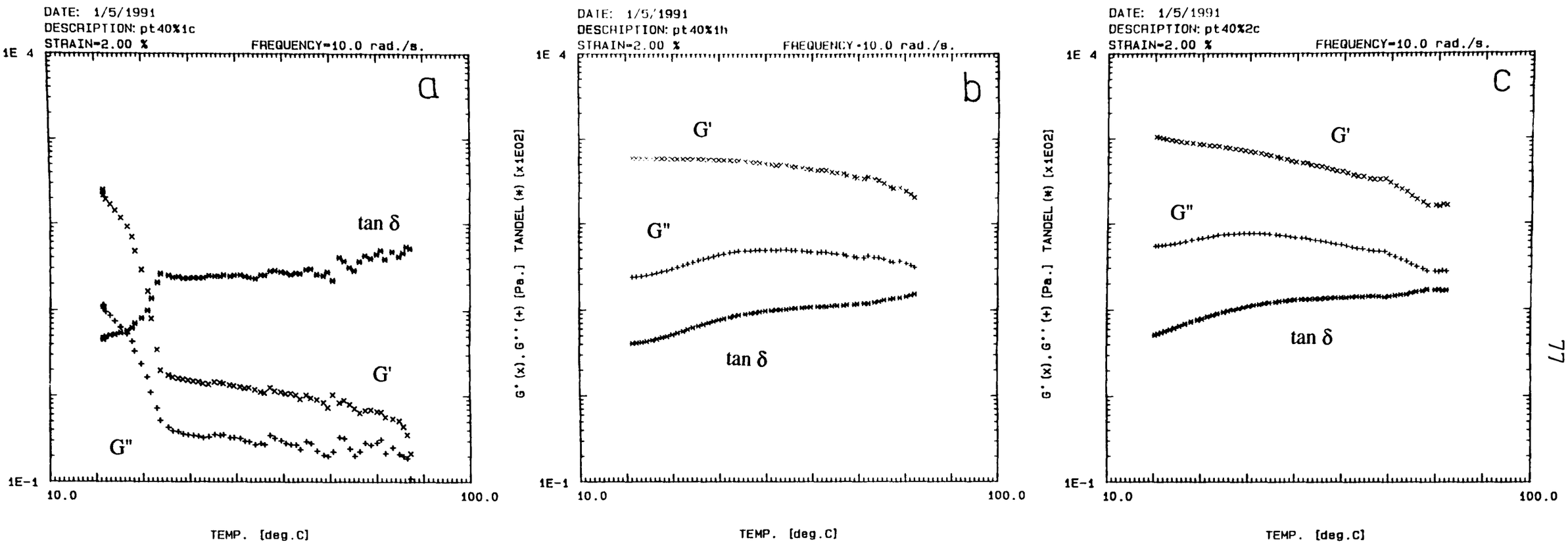


Figure 4.11 Changes in structural moduli of 1% high methoxy pectin with 40% ethylene glycol. a) first cooling, b) heating, c) second cooling.

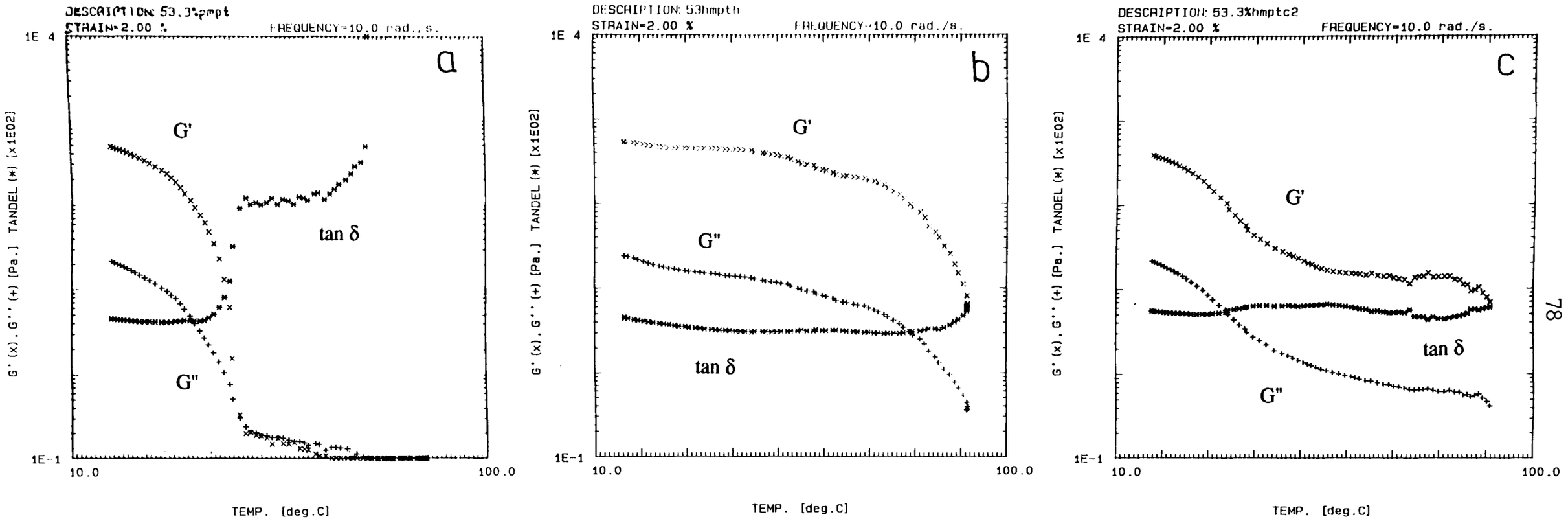


Figure 4.12 Changes in structural moduli of 1% high methoxy pectin with 53.3% ethylene glycol. a) first cooling, b) heating, c) second cooling.

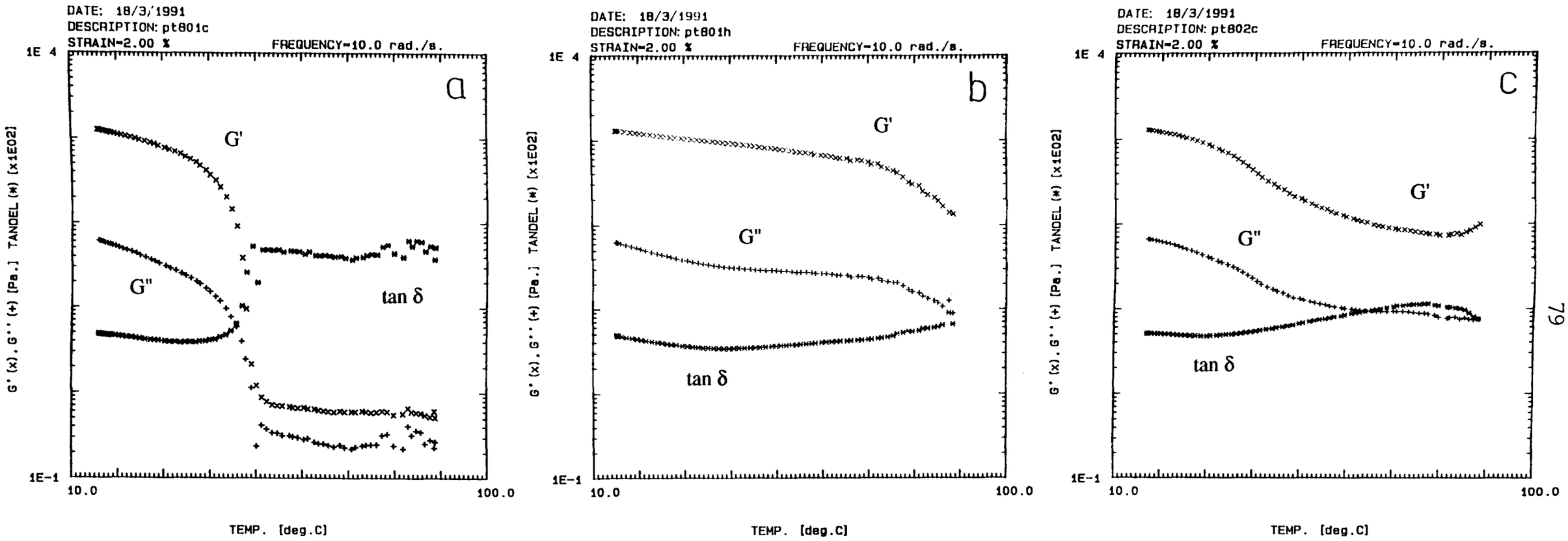


Figure 4.13 Changes in structural moduli of 1% high methoxy pectin with 80% ethylene glycol. a) first cooling, b) heating, c) second cooling.

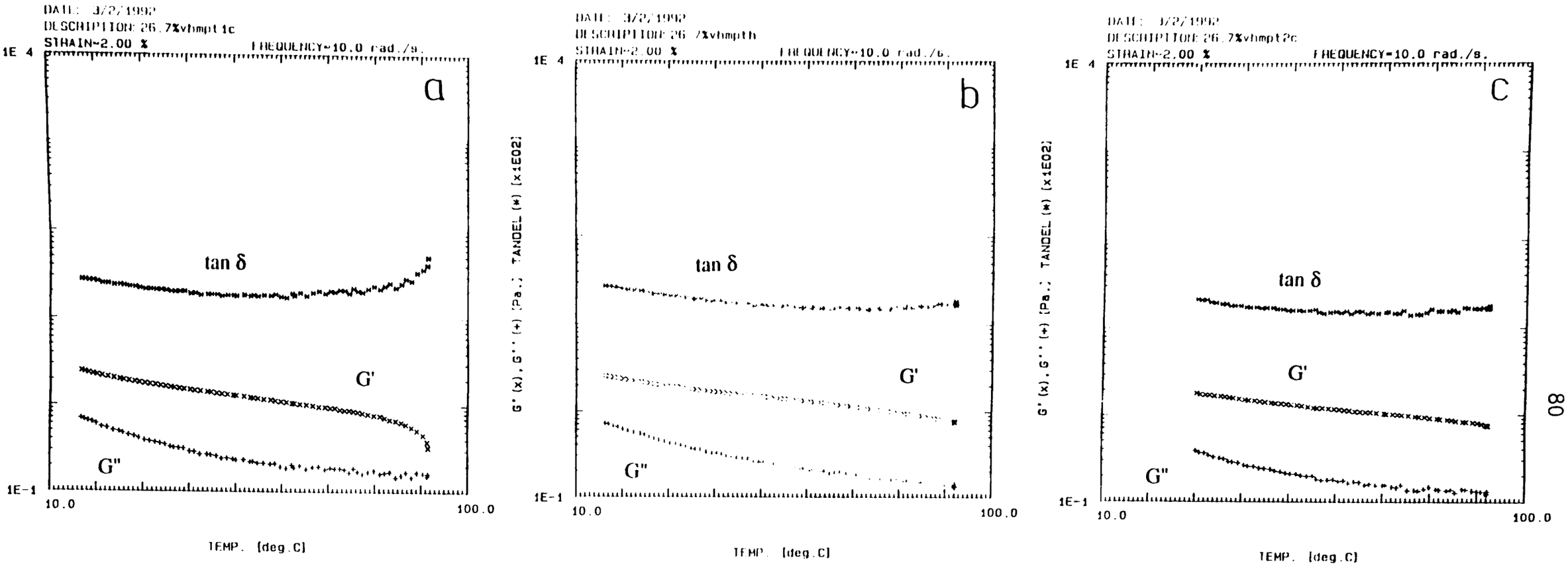
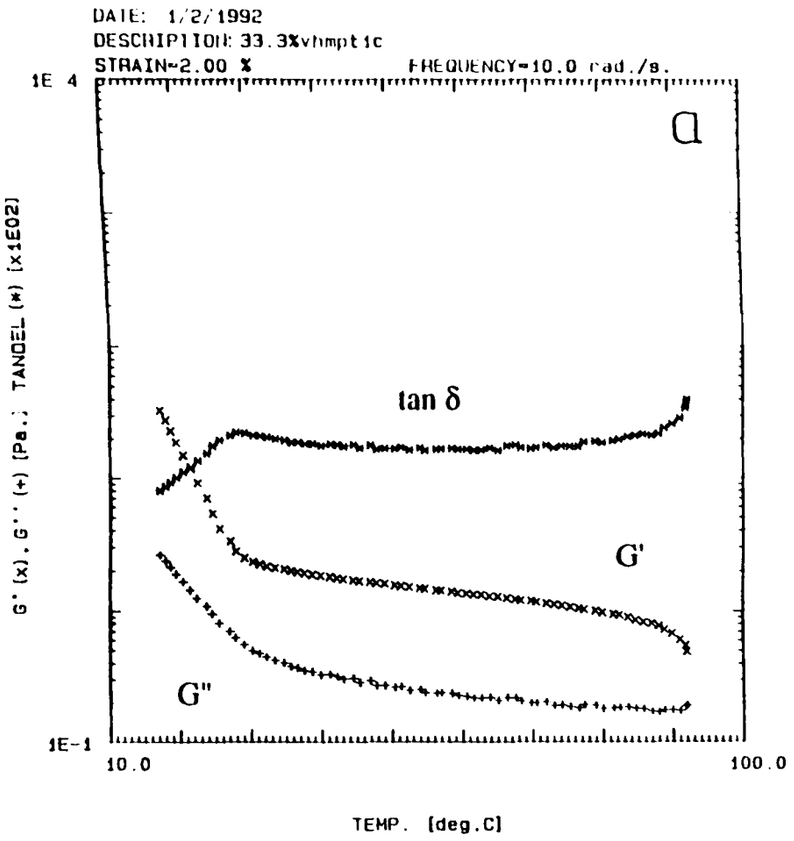
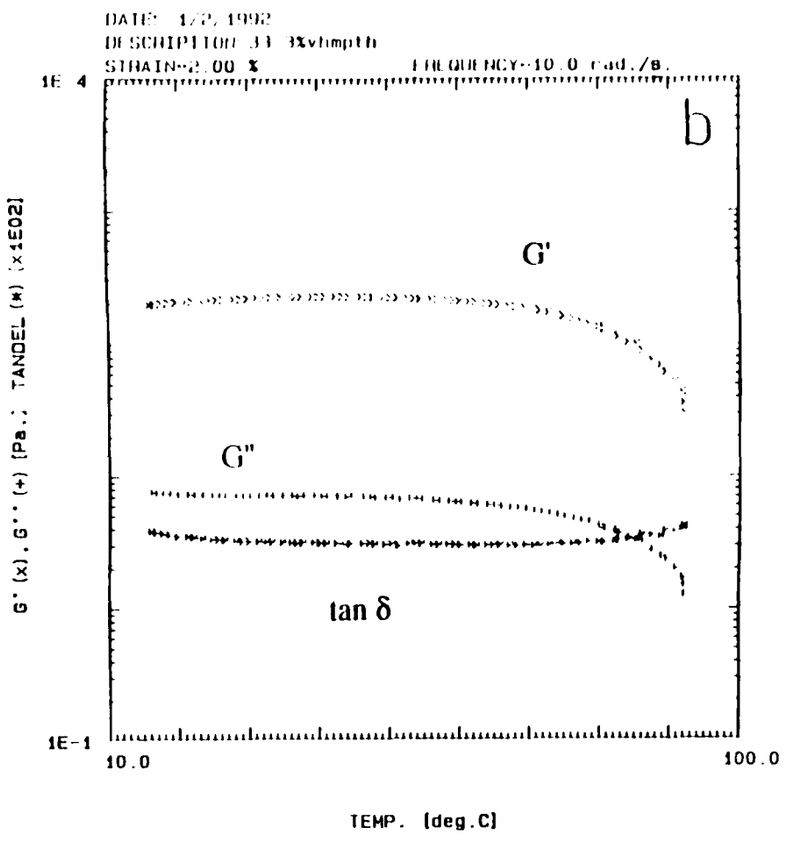


Figure 4.14 Changes in structural moduli of 1% very high methoxy pectin with 26.7% ethylene glycol. a) first cooling, b) heating, c) second cooling.





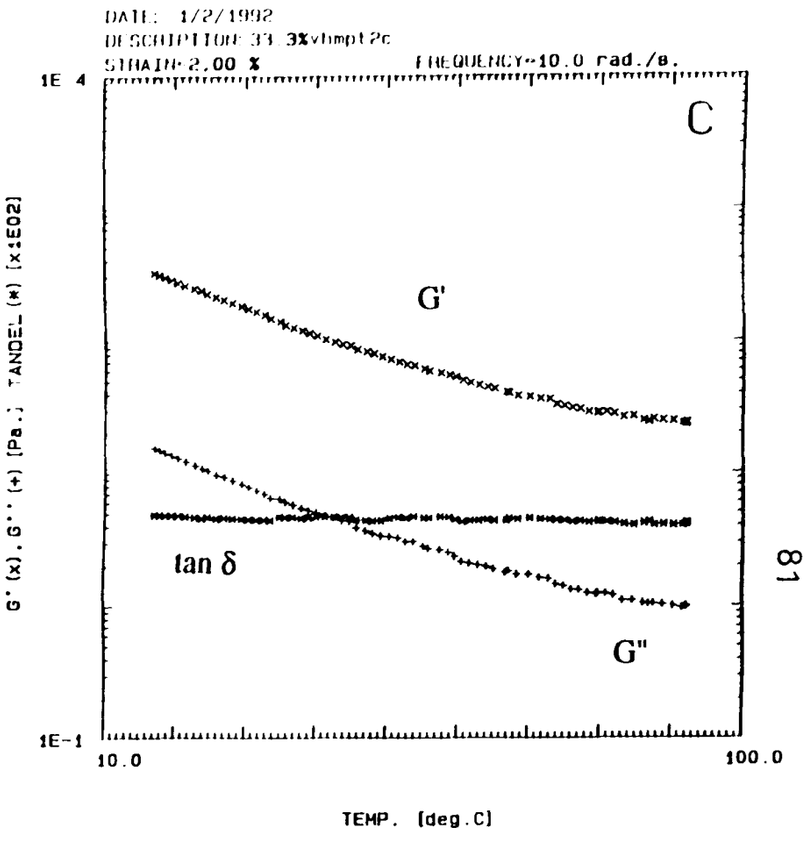


Figure 4.15 Changes in structural moduli of 1% very high methoxy pectin with 33.3% ethylene glycol. a) first cooling, b) heating, c) second cooling.

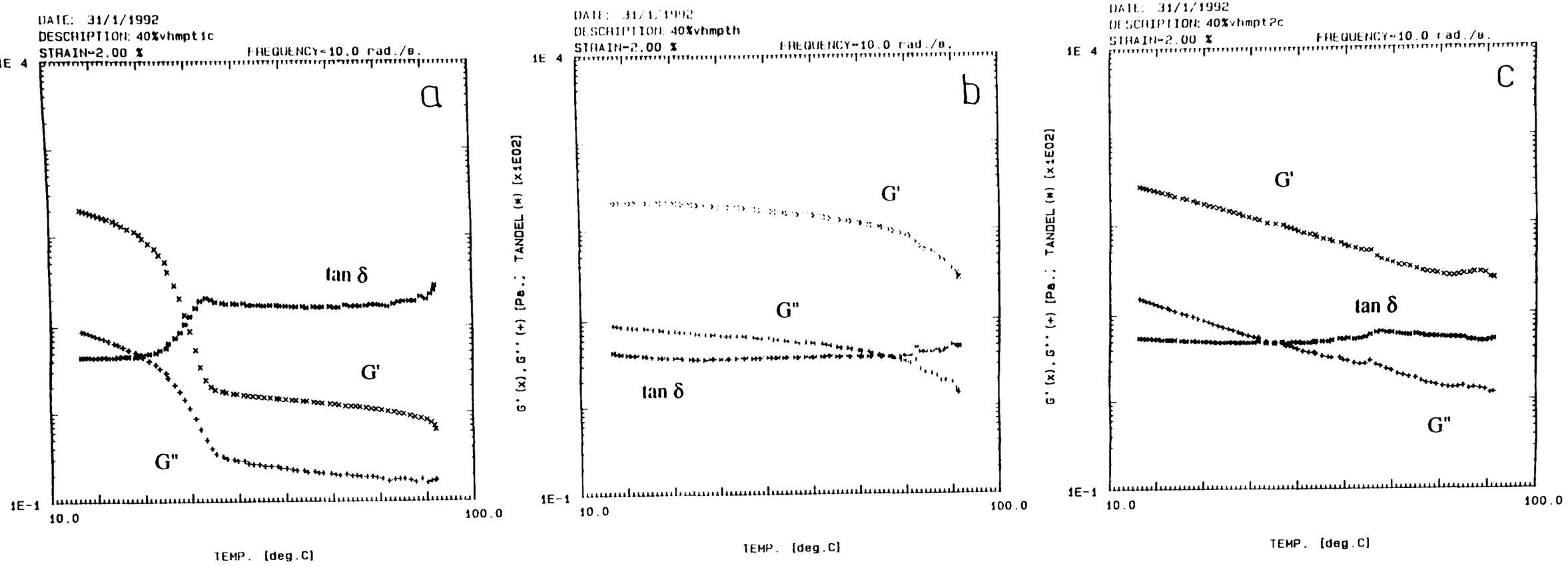
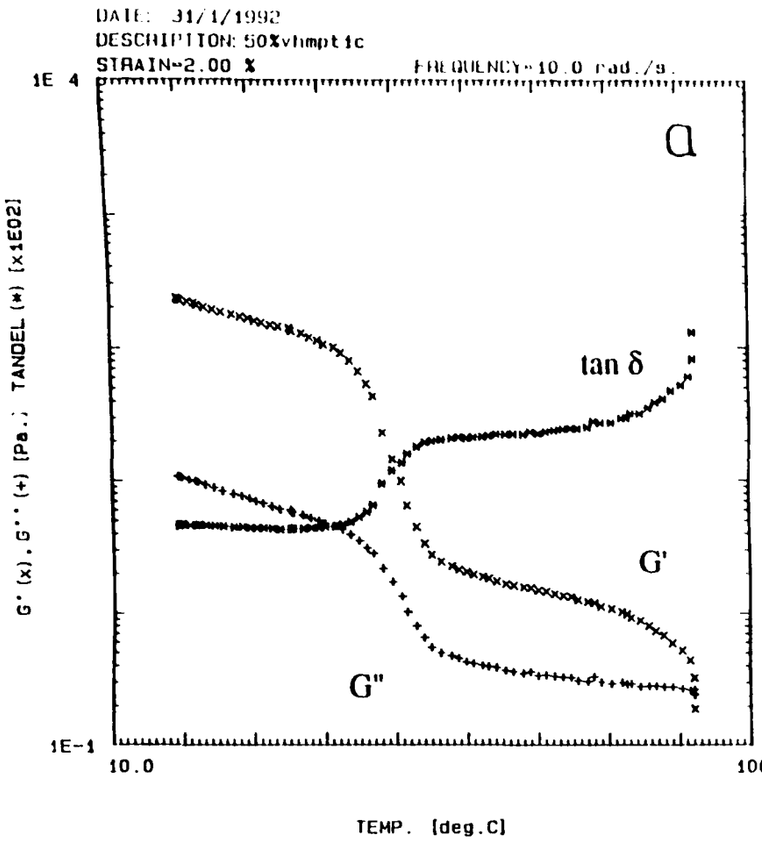
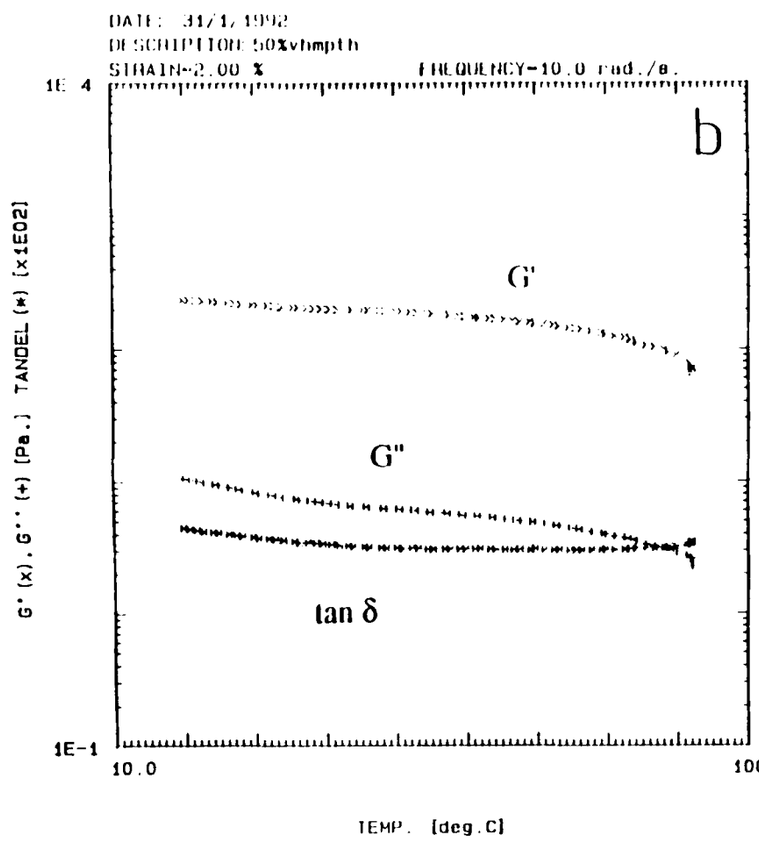


Figure 4.16 Changes in structural moduli of 1% very high methoxy pectin with 40% ethylene glycol. a) first cooling, b) heating, c) second cooling.





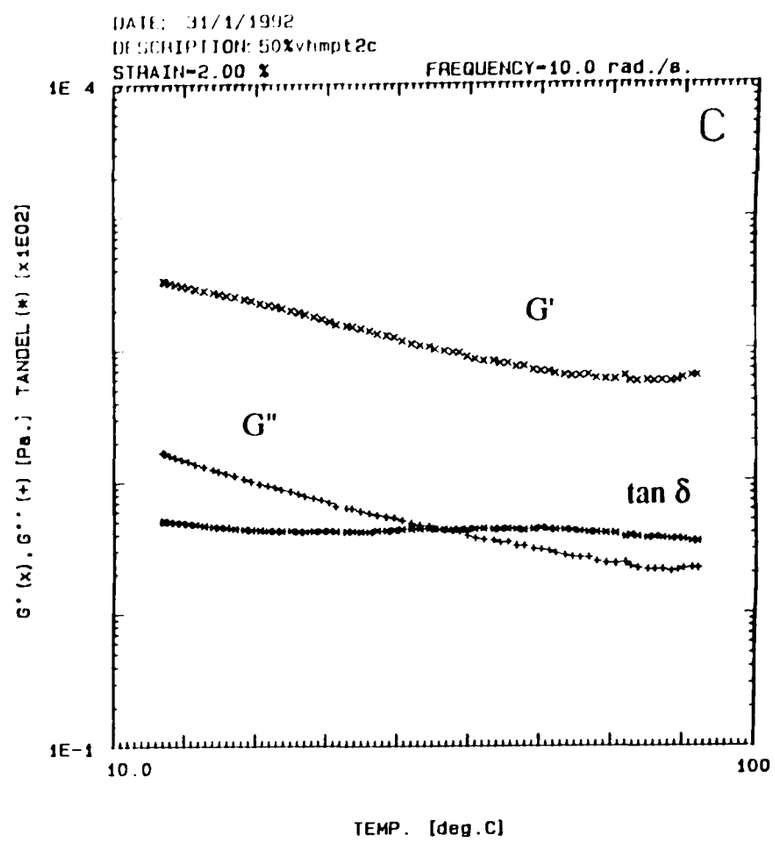


Figure 4.17 Changes in structural moduli of 1% very high methoxy pectin with 50% ethylene glycol. a) first cooling, b) heating, c) second cooling.

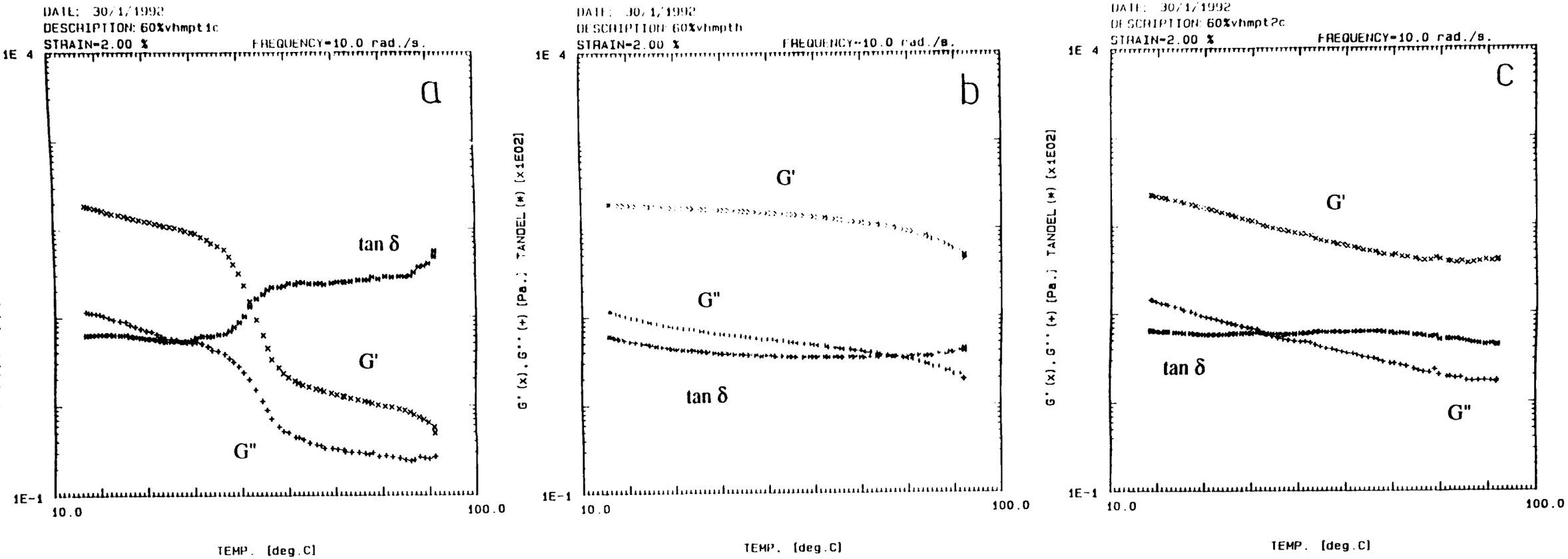


Figure 4.18 Changes in structural moduli of 1% very high methoxy pectin with 60% ethylene glycol. a) first cooling, b) heating, c) second cooling.

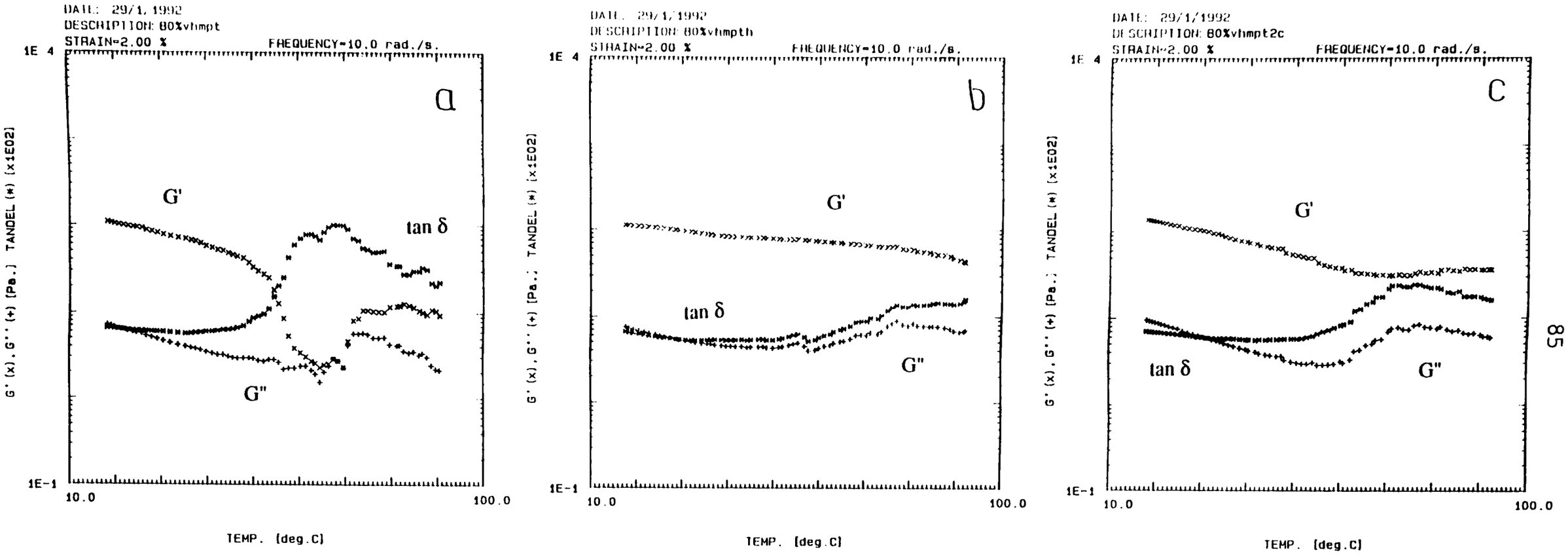


Figure 4.19 Changes in structural moduli of 1% very high methoxy pectin with 80% ethylene glycol. a) first cooling, b) heating, c) second cooling.

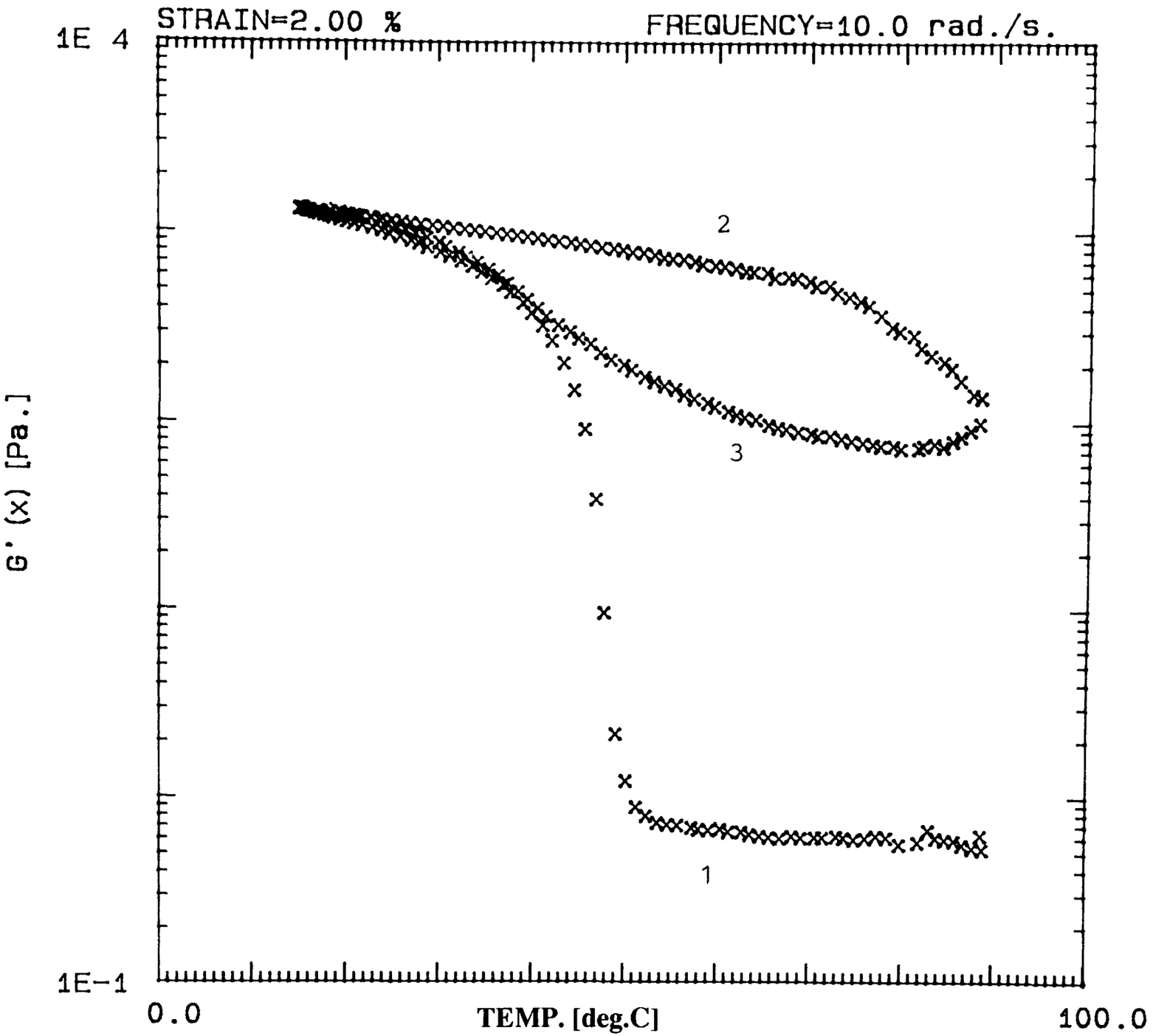


Figure 4.20 Changes in G' on 1) first cooling, 2) heating, 3) second cooling for 1% HM pectin with 80% ethylene glcyol.

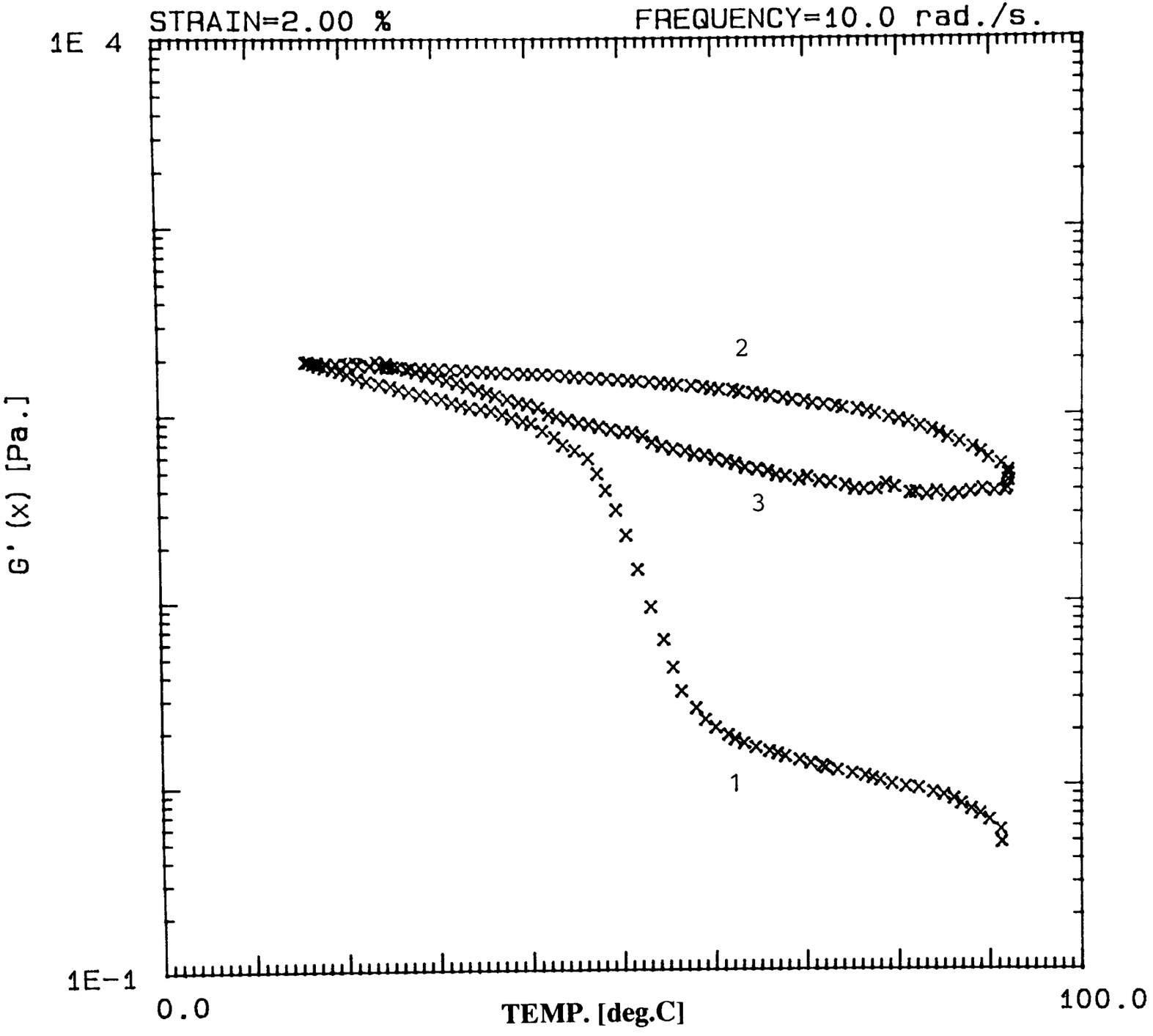


Figure 4.21 Changes in G' on 1) first cooling, 2) heating, 3) second cooling for 1% VHM pectin with 60% ethylene glcyol.

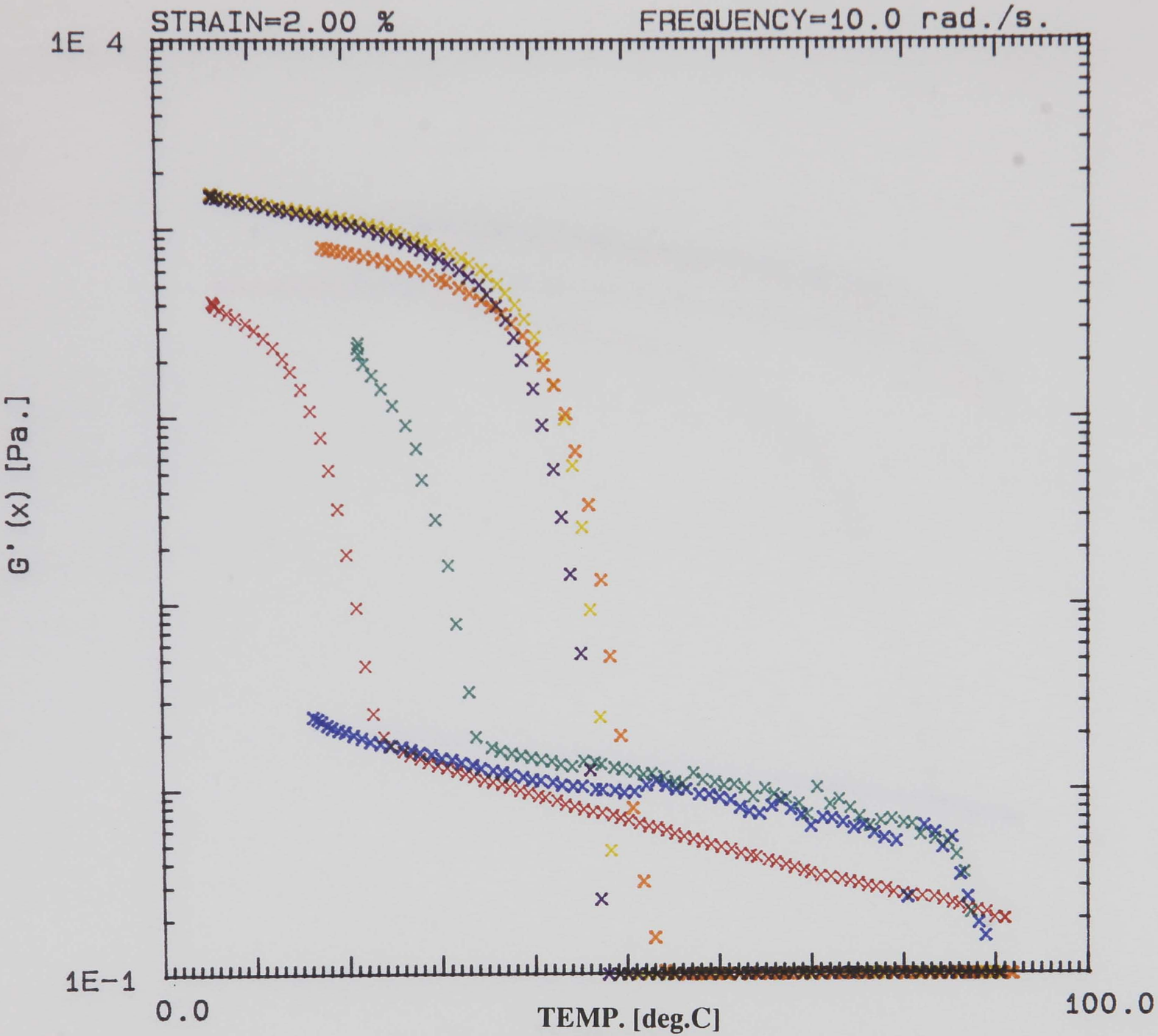


Figure 4.22 Change in G' on first cooling with increasing ethylene glycol concentration. 1% HM pectin.

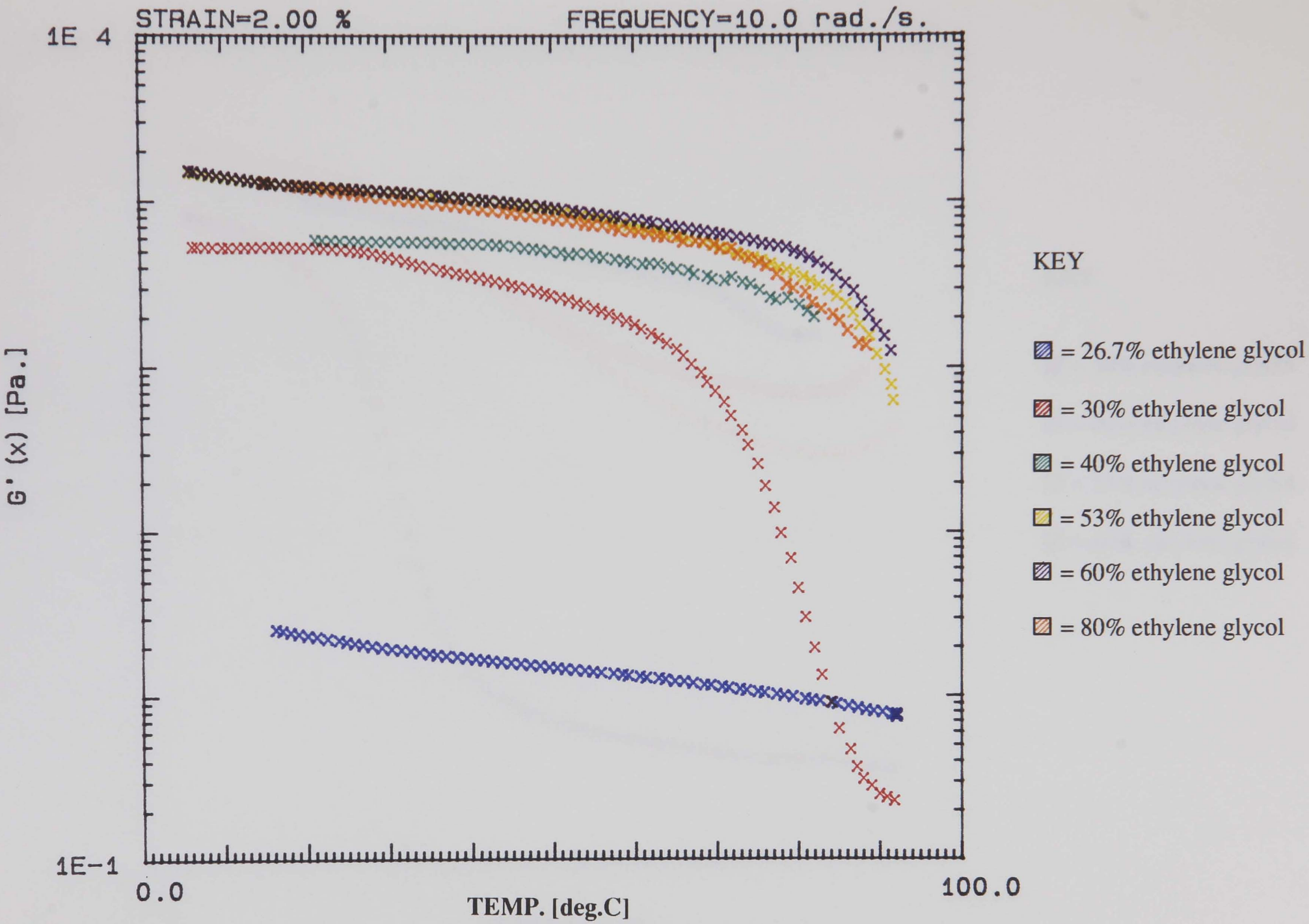


Figure 4.23 Change in G' on heating with increasing ethylene glycol concentration. 1% HM pectin.

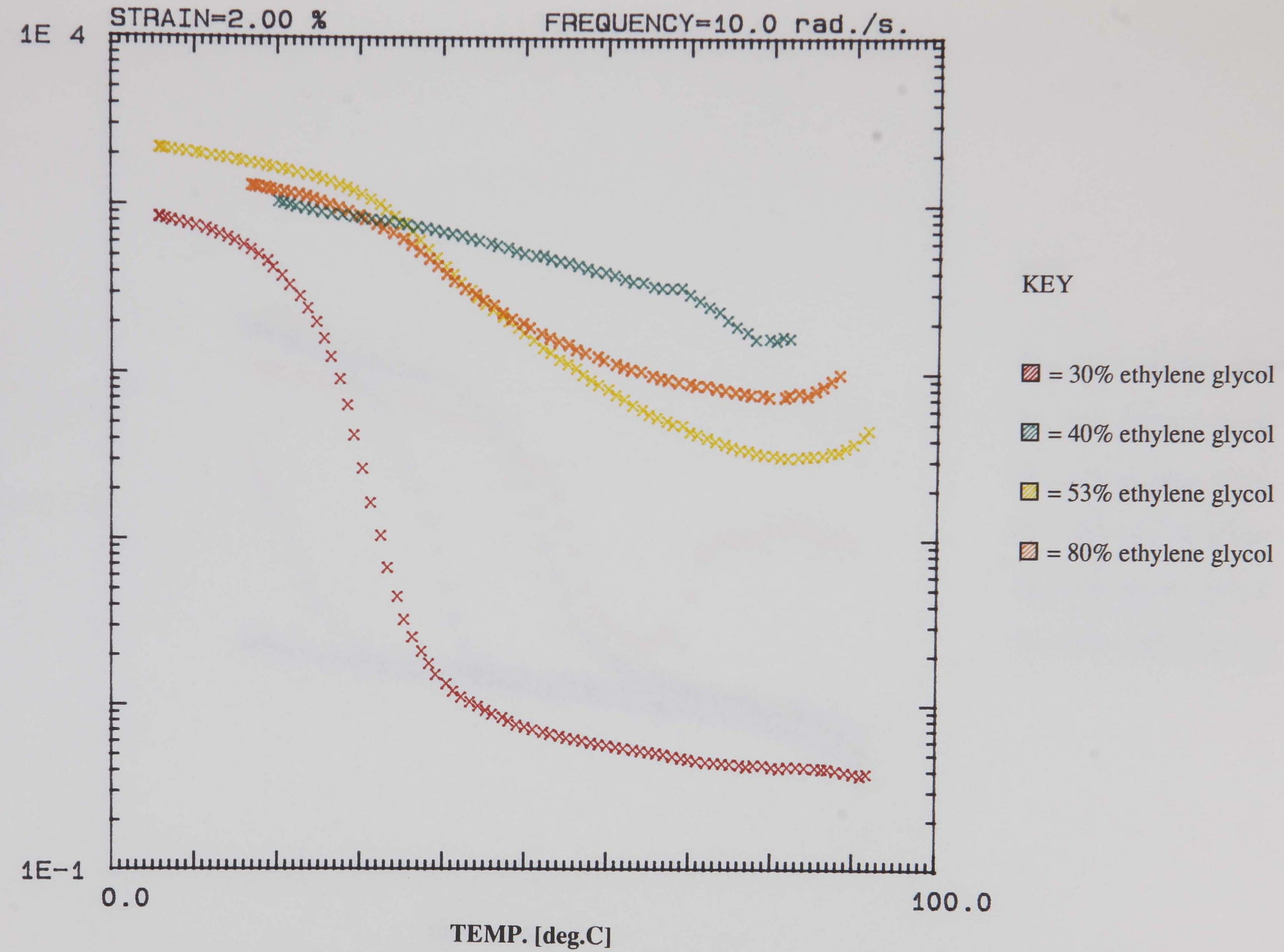


Figure 4.24 Change in G' on second cooling with increasing ethylene glycol concentration. 1% HM pectin.

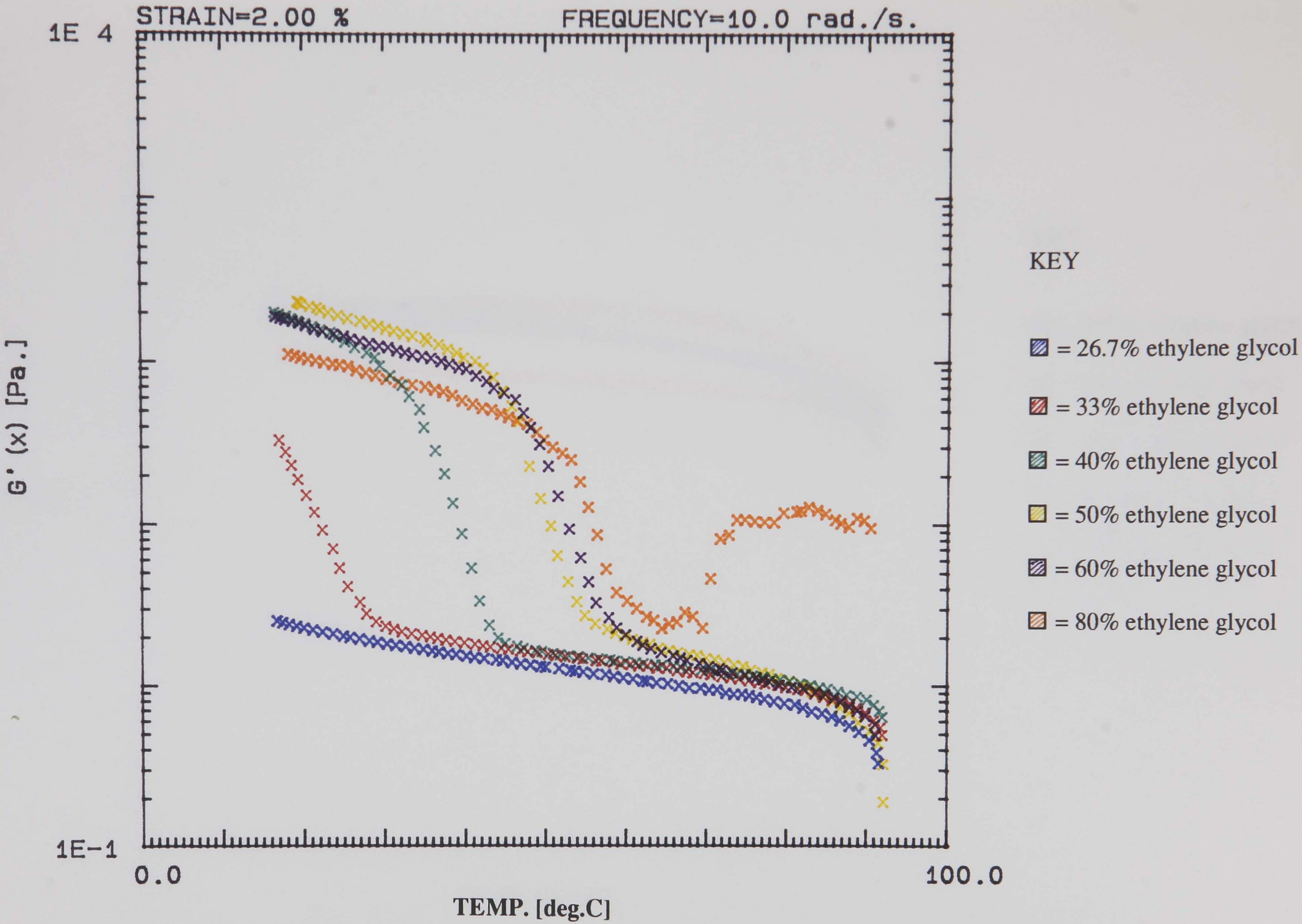


Figure 4.25 Change in G' on first cooling with increasing ethylene glycol concentration. 1% VHM pectin.

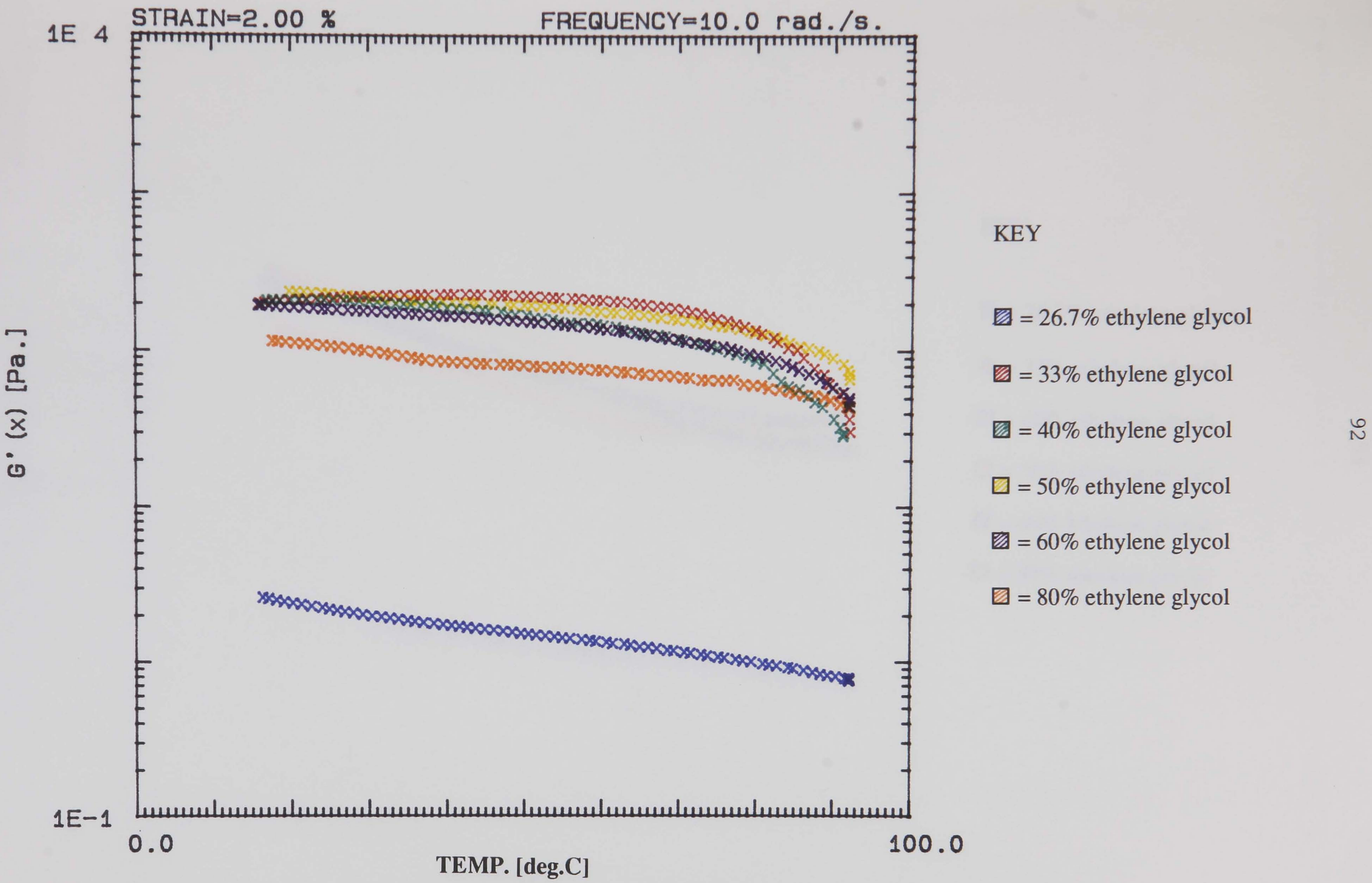


Figure 4.26 Change in G' on heating with increasing ethylene glycol concentration. 1% VHM pectin.

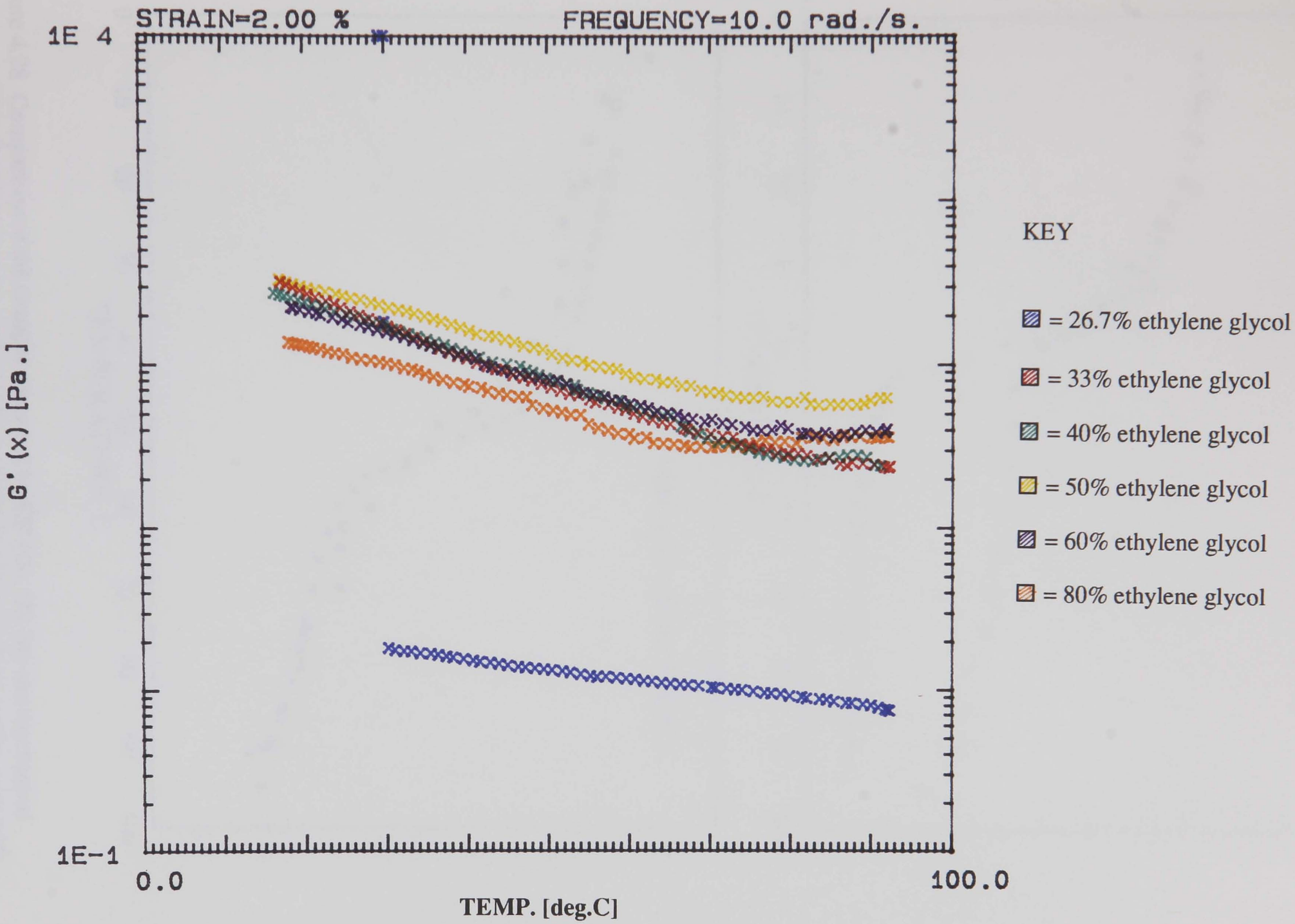
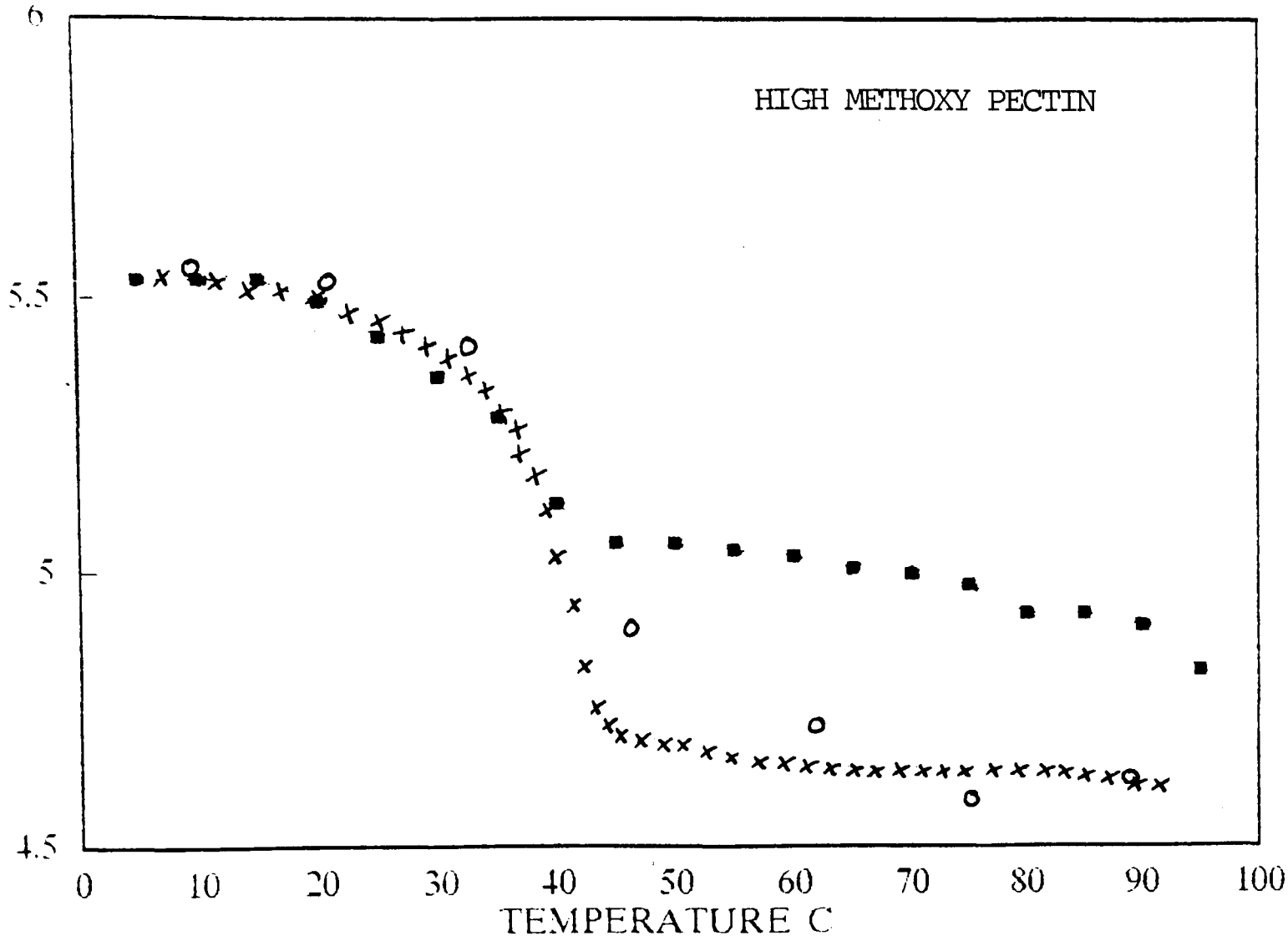


Figure 4.27 Change in G' on second cooling with increasing ethylene glycol concentration. 1% VHM pectin.



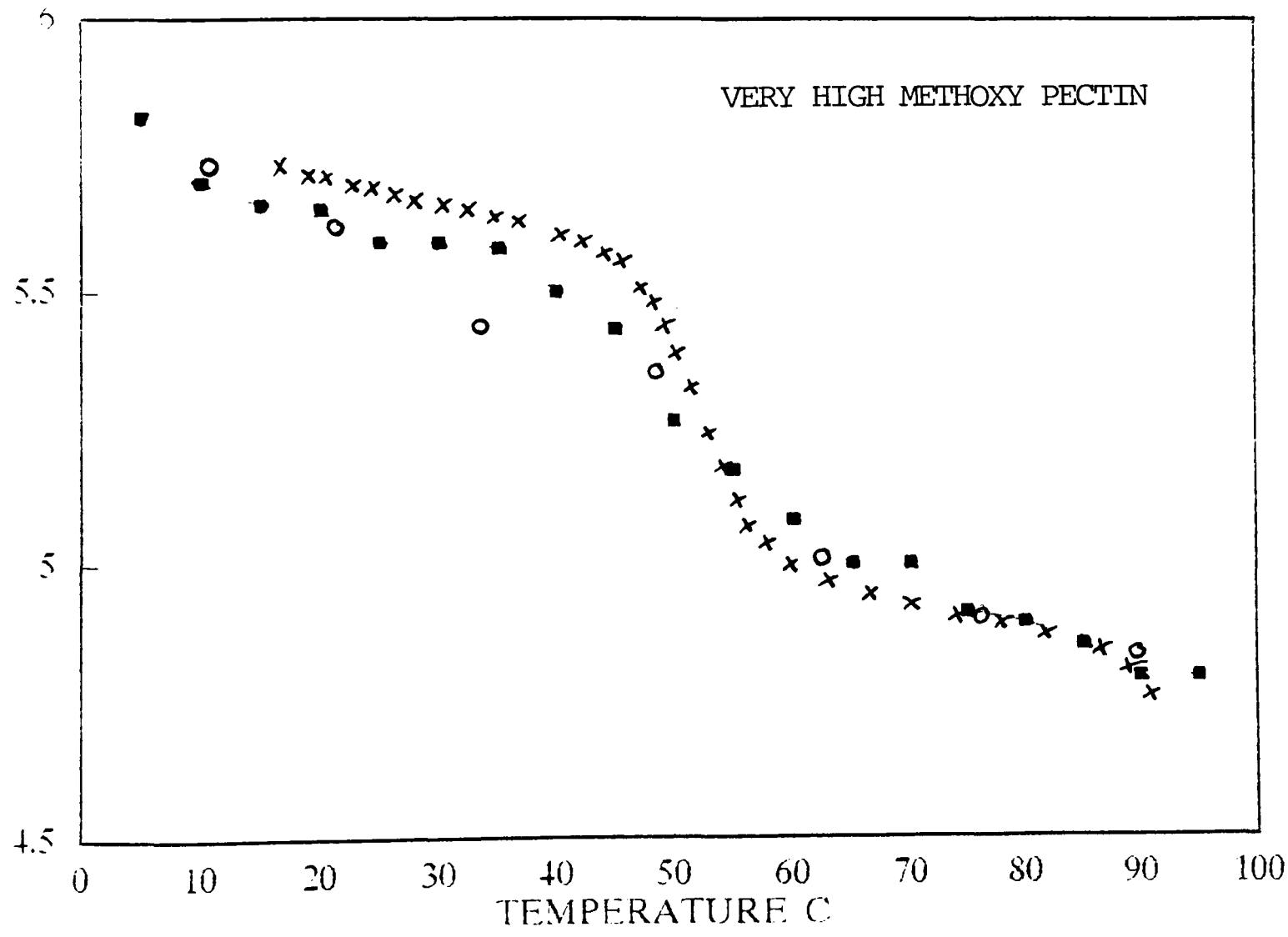


Figure 4.28 Comparison of the changes measured by CD (○), OR (■) and mechanical spectroscopy (x) for high methoxy and very high methoxy pectin on first cooling with 60% ethylene glycol.

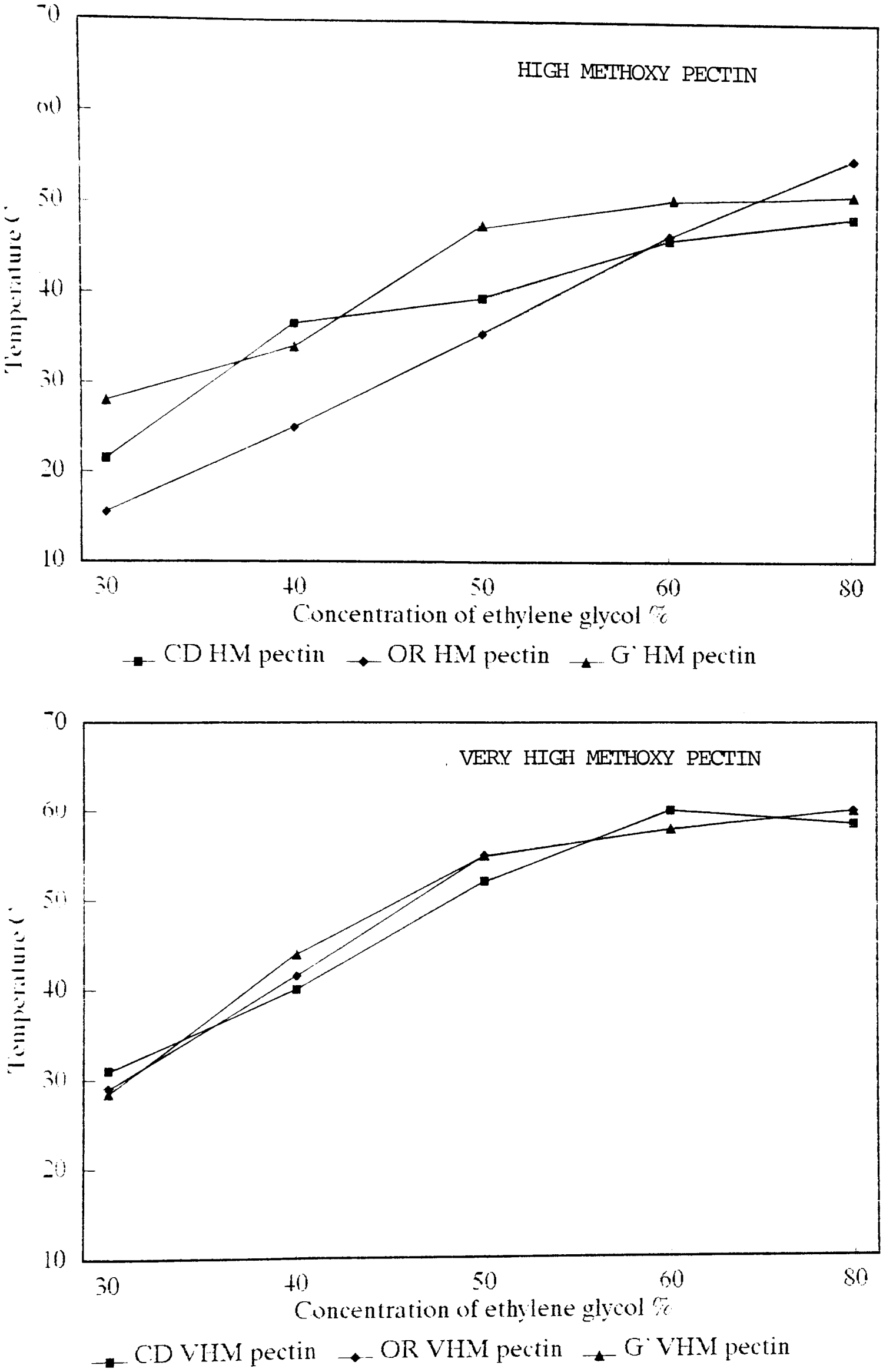


Figure 4.29 Comparison of the onset of ordering, measured by CD and OR, and gelation measured by an increase in G', for high methoxy and very high methoxy pectin with increasing ethylene glycol concentration.

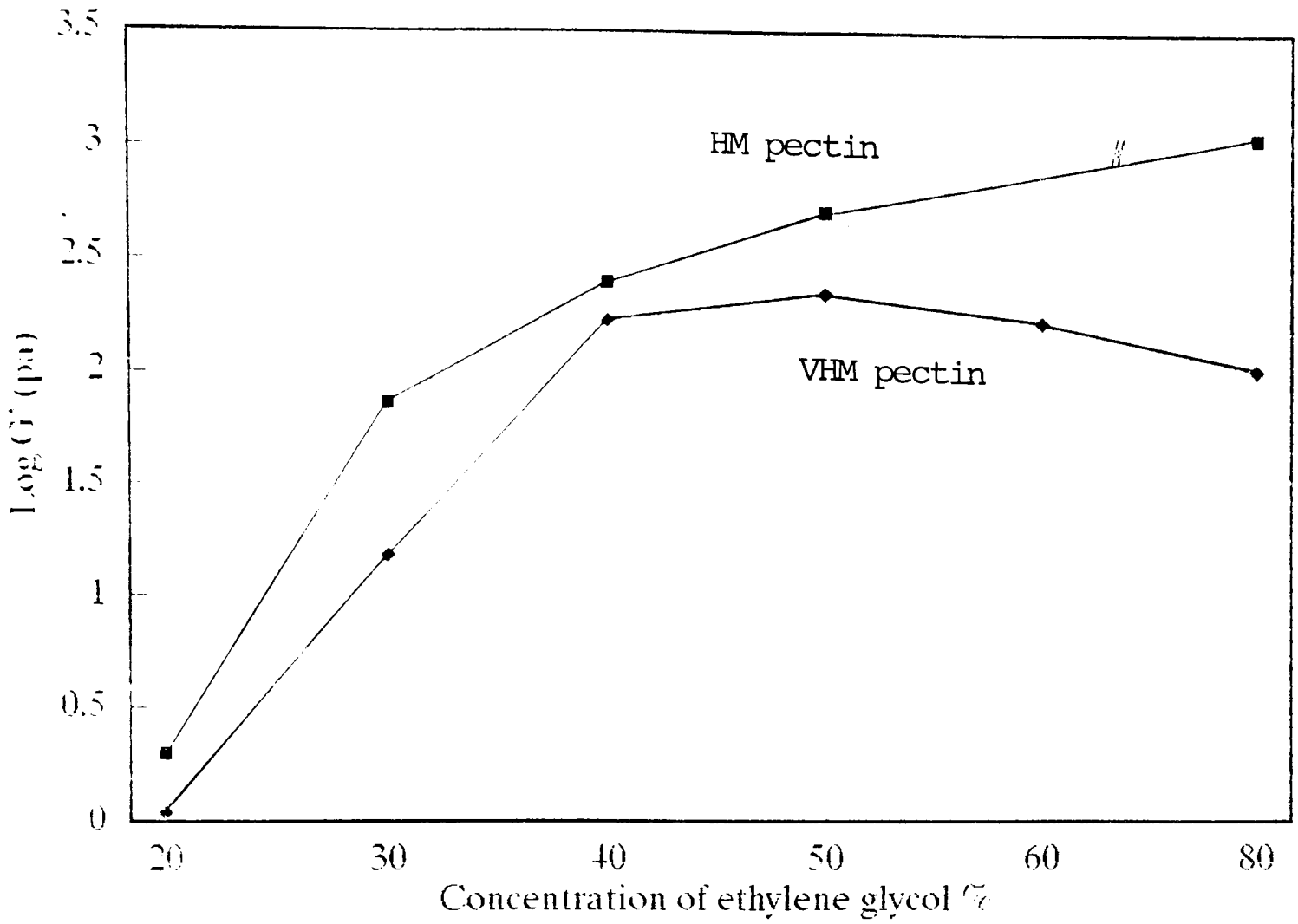


Figure 4.30 G' at 20°C during the first cooling of both high methoxy and very high methoxy pectin with increasing ethylene glycol concentration.

4.5 Response to Acid and Salt

Differences in the response of high methoxy and very high methoxy pectin to changes in solvent are also shown by their response to acid and salt in dilute solution. As detailed in Section 3.1, solutions of both were prepared in deionised water to give a viscosity at neutral pH approximately twice that of water (i.e. $\eta_{rel} \approx 2$). Ostwald Viscometry (Section 2.5) provides a simple technique of measuring polymer 'size' in solution to determine if there is any intramolecular association, which would cause the coil dimensions to diminish, or any intermolecular associations which would result in an increase in molecular size.

Four tests were carried out on each of the pectin samples (high methoxy and very high methoxy) to determine the individual, and joint effects, of acid (hydrochloric, pH2) and salt (sodium chloride, 0.5M) upon the polymer;

a) control, no acid or salt

b) addition of acid

c) addition of salt

d) addition of acid and salt

Figure 4.31 clearly shows the response of the polymer to the changes in solvent environment. Looking at the control samples for both pectins it is seen that very high methoxy pectin has a slightly lower relative viscosity than high methoxy pectin. This is an expected consequence of the difference in charge density of the polymers. The very highly esterified pectin has few 'free' carboxylic acid groups as the majority are substituted with the methoxyl group, this reduces the charge density of the polymer chains hence reducing electrostatic repulsion and allowing contraction. The lower methyl esterified pectin sample has a higher charge density and the polymer is expanded due to charge repulsion.

Acidification to pH 2 causes a substantial reduction in viscosity (indicating a compaction of the polymer coil), greater for high methoxy pectin than very high methoxy pectin. Closely similar changes were observed on addition of a high concentration of salt (0.5M NaCl) at neutral pH. Both effects can be explained by suppression of intramolecular electrostatic repulsion (by neutralisation of charged carboxyl groups by acid, or charge-screening by salt), allowing polymer coils to relax to a less expanded conformation. The lower response of very high methoxy pectin is an expected consequence of its lower content of free carboxyl groups, and the steric hindrance to coil compaction provided by the bulky methyl ester groups.

When both reagents are used together (i.e. 0.5M NaCl at pH 2), however, the reduction in viscosity is less than when either is used separately. In other words, addition of salt at low pH raises the solution viscosity. The likely interpretation is that at low pH, where the pectin chains are virtually uncharged, addition of 0.5M NaCl is sufficient to promote some limited intermolecular association, giving crosslinked species of higher molecular weight (and therefore higher viscosity) than the individual chains. As shown in Figure 4.31, the increase is far more pronounced for very high methoxy pectin than for high methoxy pectin, consistent with lower solubility in water, due to its higher content of hydrophobic (methyl) substituents.

Gelation does not take place in these samples due to the low concentration of pectin, however, the situation is directly analogous to that occurring in gelling pectin in the presence of ethylene glycol. The mechanism of limited aggregation described here provides an explanation for the structuring measured by mechanical spectroscopy, with G' greater than G", found in both pectin samples subsequent to their preparation. It also provides an in-sight into the nature of the thermo-irreversibility of highly esterified pectin gels. In the presence of acid and any water perturbing additive there will be a degree of structure retention at all temperatures.

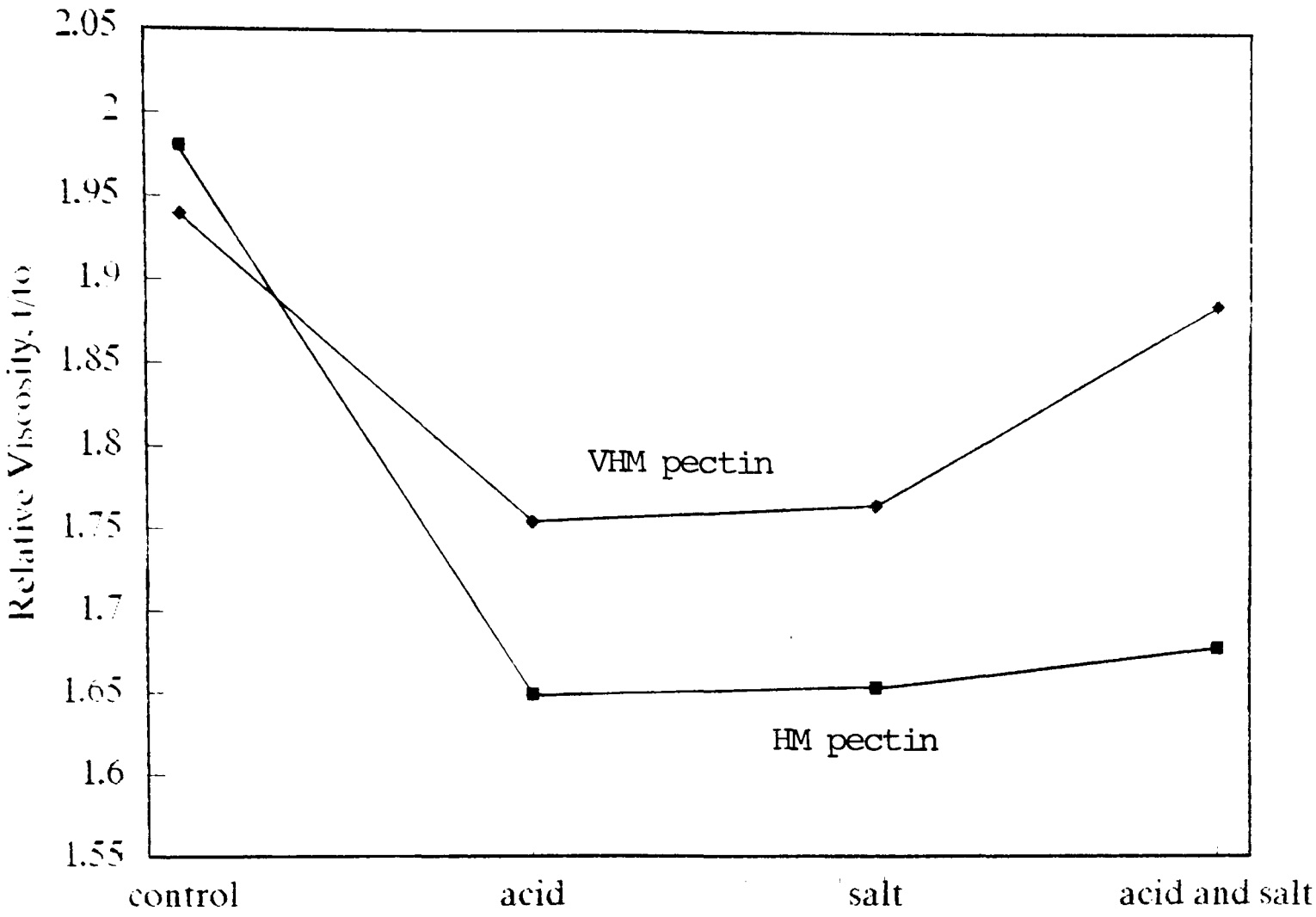


Figure 4.31 Relative viscosity of high methoxy pectin and very high methoxy pectin in the presence of hydrochloric acid (pH 2) and sodium chloride (0.5M).

4.6 Discussion

Very high methoxy pectin, under conditions of high ethylene glycol, shows a reduction in resistance to small deformation (fall in G', Figure 4.30) and loss of the self-supporting network as observed visually, however, chiroptical measurements, illustrate that the ordered structure is still formed (and indeed is more stable, as judged by the progressive increase in temperature at which it forms, Figure 4.29). This evidence strongly indicates the involvement of two processes in the formation of these highly esterified pectin gels. Walkinshaw and Arnott (1981b) have shown, by X-ray diffraction studies of pectin in the condensed state, the existence of 3_1 helices under all experimental conditions studied (different ester content; different salt conditions). It is suggested that this ordered structure in solid pectin is also found in the ordered conformation of the gelled state. Gelation would then proceed by association of these ordered chains to form the gelled network.

The coincidence of the development of the macroscopic network (increase in G') with the conformational change indicated by OR and CD, Figures 4.28 and 4.29, indicates that the process of ordering occurs almost simultaneously with chain association. The most likely interpretation of the mechanism of the two processes in gelation is:

Stage 1: formation of 3_1 structure (as for X-ray evidence)

Stage 2: aggregation of 3-fold helices to give gel junctions (immediately following, or coincident with, conformational ordering).

The onset of the transition of conformational ordering increases with increasing ethylene glycol concentration for both series of pectins, however, there is a reduction in gelling ability of very high methoxy pectin with high concentrations of the co-solute. This leads to the conclusion that ethylene glycol promotes stage 1 of the gelation process, but is antagonistic to stage 2.

The X-ray evidence of Walkinshaw and Arnott (1981b) indicates two sources of conformational stability for pectin in the condensed state:

1) hydrogen bonding between OH groups on contiguous 3_1 helices, giving trigonal trimeric assemblies.

2) 'hydrophobic' clustering of methyl groups between the trimeric structures, again arranged trigonally.

This gives rise to the proposed ordered packing arrangement for condensed pectin illustrated in Figure 4.32.

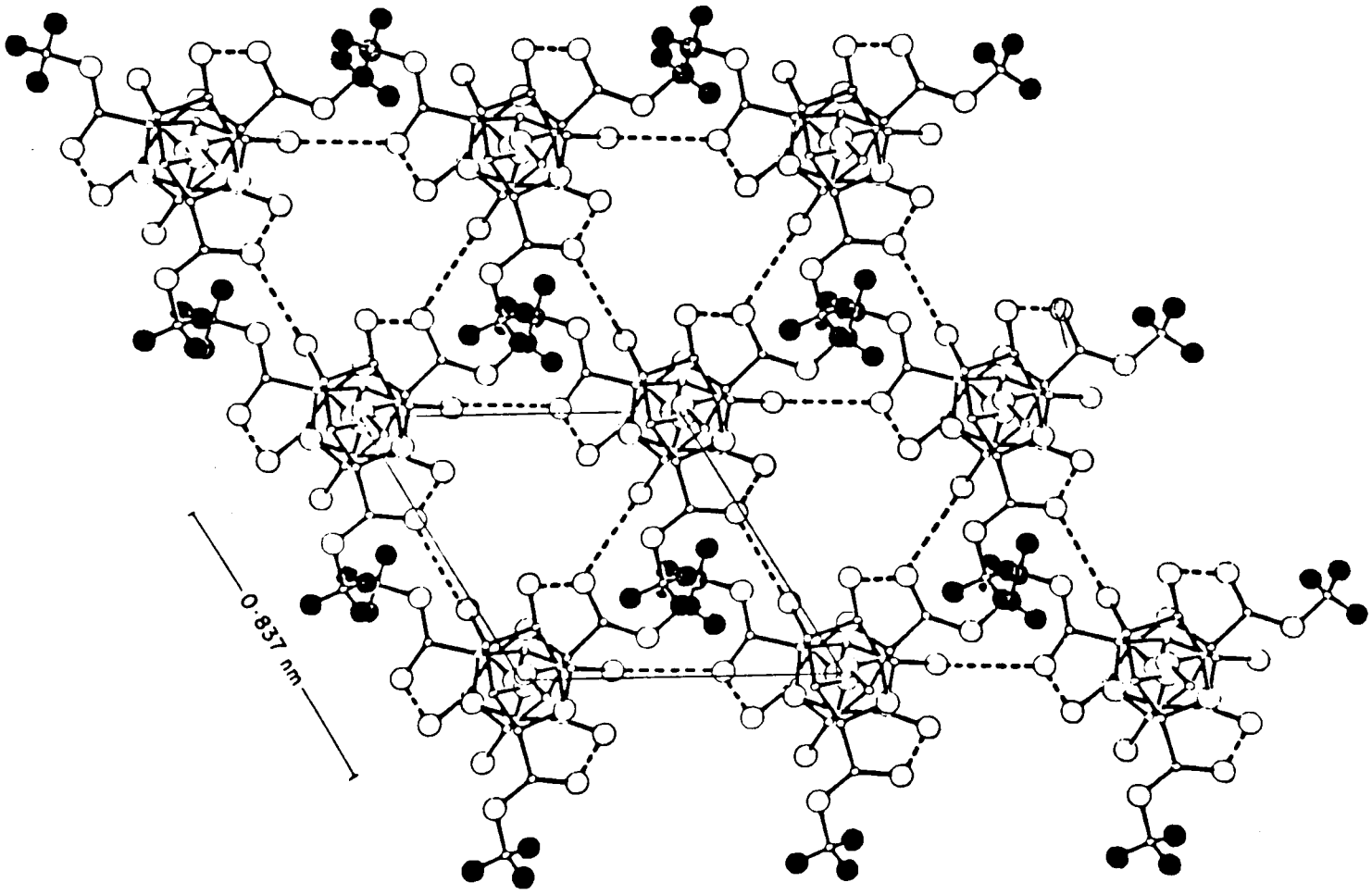


Figure 4.32 A view of the pectinic acid structure (DE = 100%) along the (001) direction. Parallel chains are packed in a hexagonal lattice. Methyl hydrogens are denoted by filled circles to emphasise the columnar stacking of methoxyl groups (From Walkinshaw and Arnott, 1981b).

It is proposed that the amphiphilic polymer (containing both polar OH groups and non-polar methyl groups) gains conformational stability in the gel state by employing the two mechanisms of association outlined above. The increased stability conferred by 'hydrophobic' interactions between methyl groups in one set of triangular channels leads to the augmentation of hysteresis evident (CD, OR and G') in the very high methoxy pectin samples during thermal cycling.

In high methoxy pectin with relatively few hydrophobic substituents, replacement of water by ethylene glycol promotes self-association of the polysaccharide chains leading to the formation of the ordered structure and subsequent gelation. Ethylene glycol is a poor solvent for the hydrophilic polymer chains. This can be rationalised by its lesser ability to form polymer-solvent hydrogen bonds in competition with polymer-polymer hydrogen bonding between OH groups of the polysaccharide chains. However, it is a better solvent than water for hydrocarbons and related hydrophobic substances (Tanford, 1980, Sinanoglu and Abdulnur, 1965) and would therefore be expected to solubilise the hydrophobic methyl groups. Evidence from the loss of gelation of the very high methoxy pectins indicates that this is so. The very highly esterified pectin contains large numbers of hydrophobic methyl groups which, when "dissolved" in ethylene glycol are no longer able to participate in hydrophobic clustering which is a requisite of gel stability. Ethylene glycol will also promote hydrogen bonding between OH groups of the polymer chains to form the fundamental ordered subunits due to the inability to form polymer-solvent interactions.

The likely (minimum) interpretation is, therefore, that the process observed chiroptically is the formation of the trigonal assemblies of 3_1 chains, but that formation of a strong network requires further association of these fundamental subunits by hydrophobic clustering of methyl groups (the mechanism of which will be discussed in greater detail in the following chapter).

Very high methoxy pectin would be expected to show greater sensitivity to ethylene glycol both in the formation of "hydrophilic" associations (stage 1) and also in the loss of "hydrophobic" associations (stage 2), as observed. The higher content of methyl groups would reduce the solubility in water therefore making it easier for ethylene glycol to promote formation of the ordered structure, stage 1, (similar behaviour is observed when the solvent quality is reduced by concentrated NaCl when the polymer is acidified at pH2). However, there are also more hydrophobic methyl groups to 'dissolve' in ethylene glycol (at the surface of the fundamental structural units) causing a loss of overall gel structure at high concentrations of ethylene glycol.

The concepts developed here will be applied in the next chapter to polysaccharides with a higher content of hydrophobic substituents.

Chapter 5: RESULTS AND DISCUSSION OF CELLULOSE DERIVATIVES

5.1 Introduction

The previous chapter described the interplay between the content of methyl groups in pectin and the response of its gelation behaviour to changes in solvent quality. In this chapter the investigation is extended to polysaccharides with a much higher content of hydrophobic substituents: methylcellulose and hydroxypropylmethylcellulose (HPMC). As detailed in Section 1.3, these share the unusual property of forming gels on heating and reverting to the solution state on cooling.

In the present work, the temperature course of the formation and loss of the gel structure has been monitored rheologically by low-amplitude oscillatory measurements (Section 2.6) and the underlying changes in molecular organisation have been probed by differential scanning calorimetry (DSC; Section 2.4). As in the studies of pectin gelation reported in Chapter 4, the work has centred on the response to ethylene glycol as co-solute. A brief comparative study was also made using a larger polyhydroxylic co-solute, sucrose, and is reported first.

5.2 Effect of Sucrose on Thermogelation of Methylcellulose

The samples were prepared from a 3% stock as detailed in 3.2, and various concentrations of 50% w/w sucrose added. DSC scans were carried out at a scan rate of 0.5 degrees per minute with heating from 5°C to 95°C and cooling back to 5°C.

As shown in Figure 5.1, the gelation of methylcellulose on heating is accompanied by a sharp endotherm in DSC. A corresponding exothermic change is observed on dissociation of the gel network (offset to lower temperatures) on cooling, but with clear evidence of the involvement of two processes which are not resolved in the heating direction. The nature of the underlying molecular events is discussed in detail in a later section (5.3.3)

Closely similar transitions were observed in the presence of sucrose at concentrations of 5, 10 and 15% w/w (Figures 5.2 to 5.4), but displaced to progressively lower temperatures. The co-operativity of the process, as measured by the peak width at half peak height (giving an indication of the number of residues undergoing conformational change at any one time) remains the same. As shown in Figure 5.5, the heating endotherm and cooling exotherm are displaced to the same extent, with the thermal hysteresis between them remaining constant at ~33°C. The enthalpy change also remains unaffected (Figure 5.6) at $\Delta H \approx 11 \pm 1$ J/g, with good agreement between the values obtained for the single endotherm obtained on heating and the bimodal exotherm on cooling.

The likely explanation of this simple displacement of both gelation and dissociation, with no evidence of any other, more specific, effect, is that the addition of sucrose lowers the solvent quality of water by forming hydrogen bonds with the water molecules. This reduces the ability of water to "dissolve" the polymer so promoting polymer-polymer interactions in preference to polymer-solvent, and therefore allowing the gel network to form at lower temperatures than would otherwise be required. Any additive that decreases the solvent power of water for the polymer would be expected to have a similar effect on the gelation

temperature. As described below, however, ethylene glycol affects the system in a quite different way, and, as in the discussion of pectin (Section 4.6), can perhaps more appropriately be regarded as a "co-solvent" (for the hydrophobic substituents of the polymer) rather then as a co-solute.

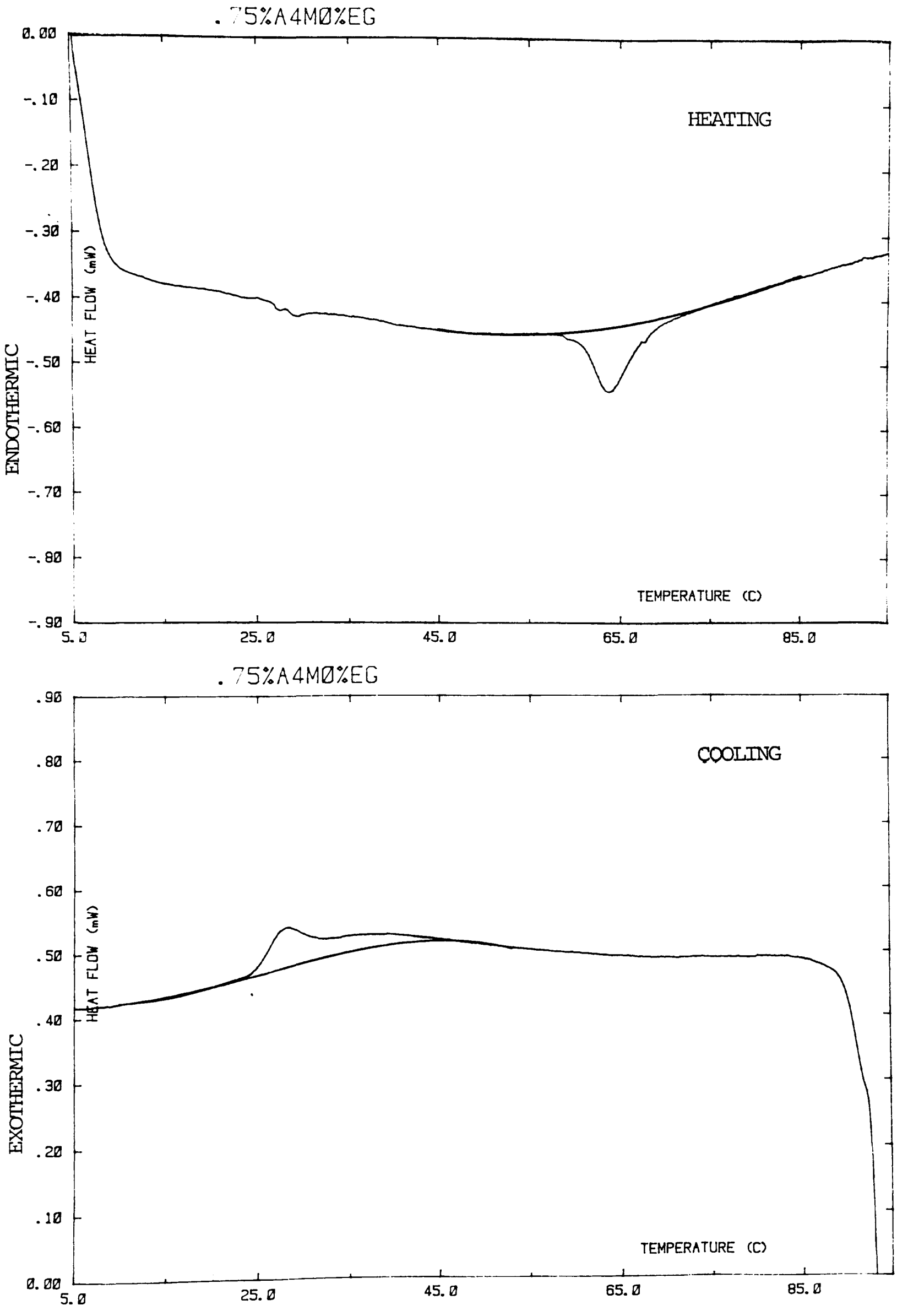


Figure 5.1 DSC of 0.75% A4M in water. Scan rate 0.5°C/min.

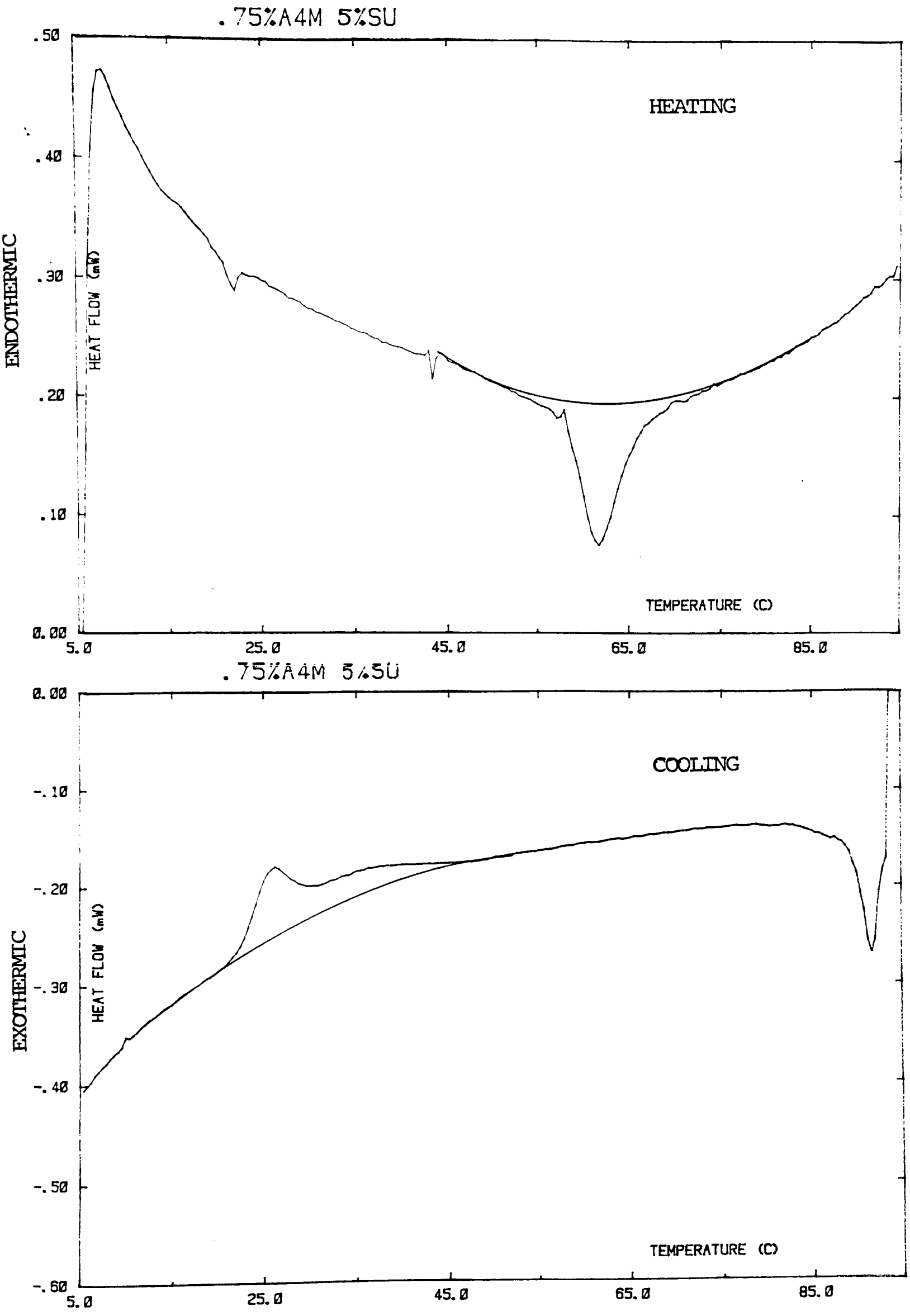


Figure 5.2 DSC of 0.75% A4M in 5% sucrose. Scan rate 0.5°C/min.

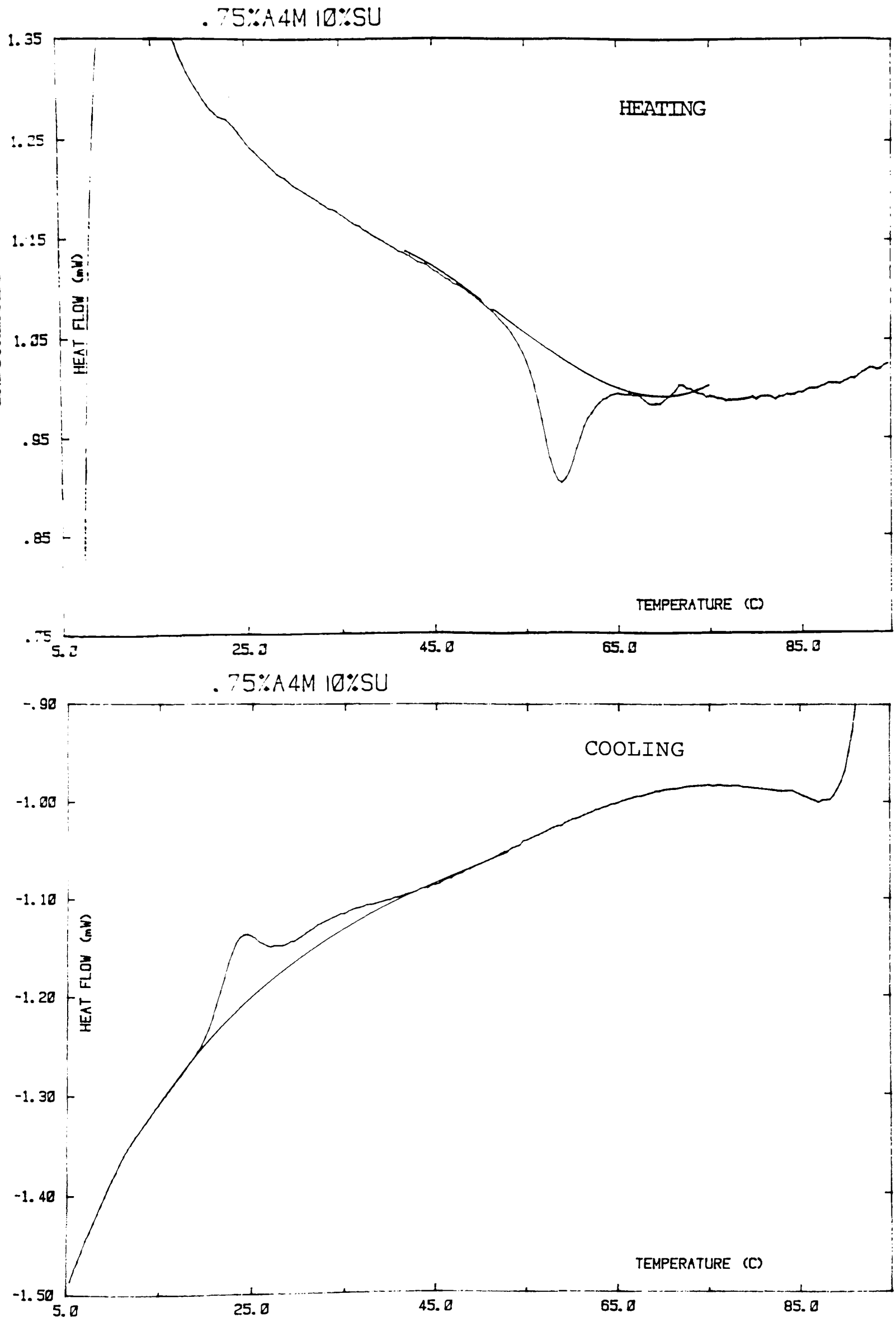


Figure 5.3 DSC of 0.75% A4M in 10% sucrose. Scan rate 0.5°C/min.

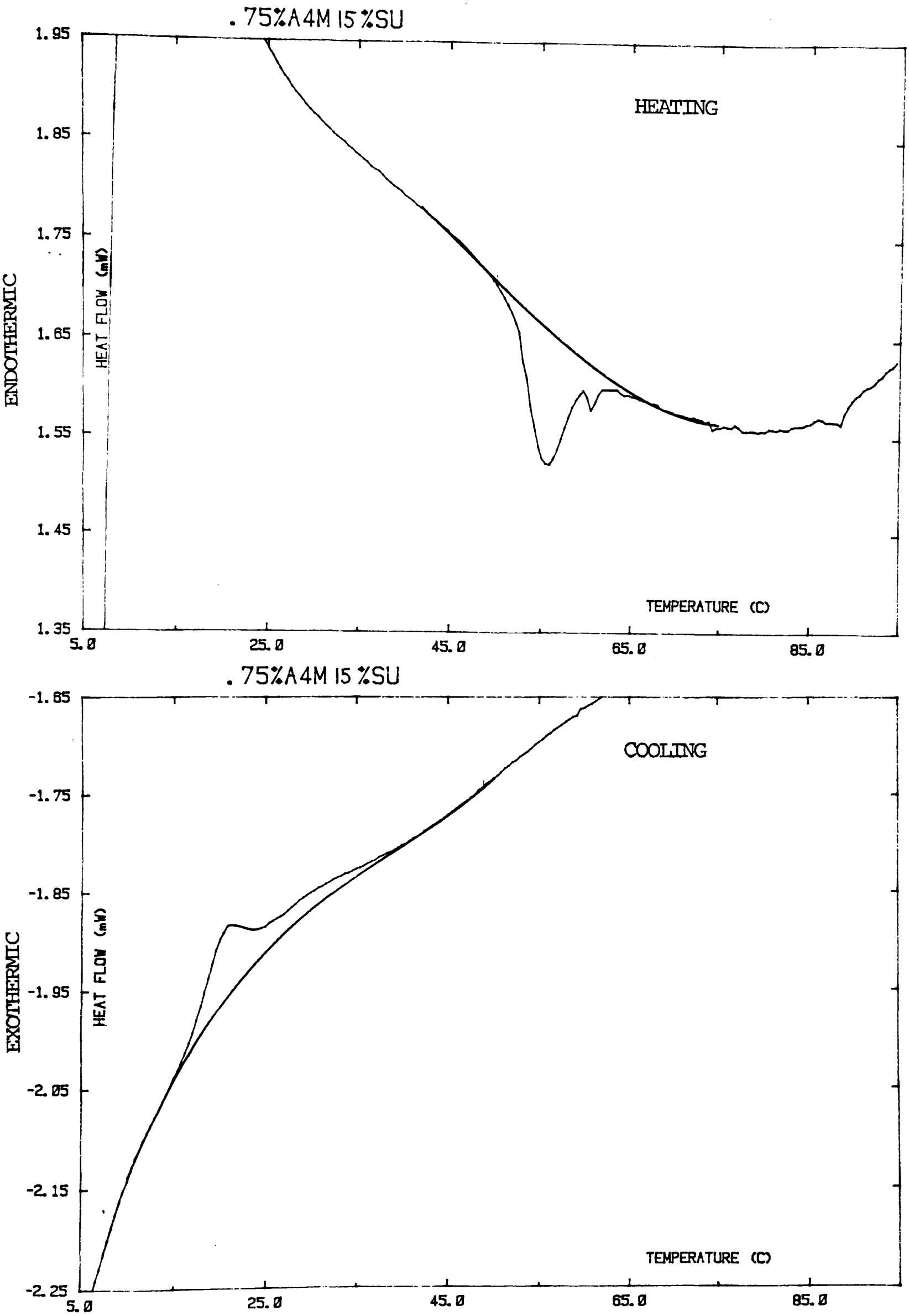


Figure 5.4 DSC of 0.75% A4M in 15% sucrose. Scan rate 0.5°C/min.

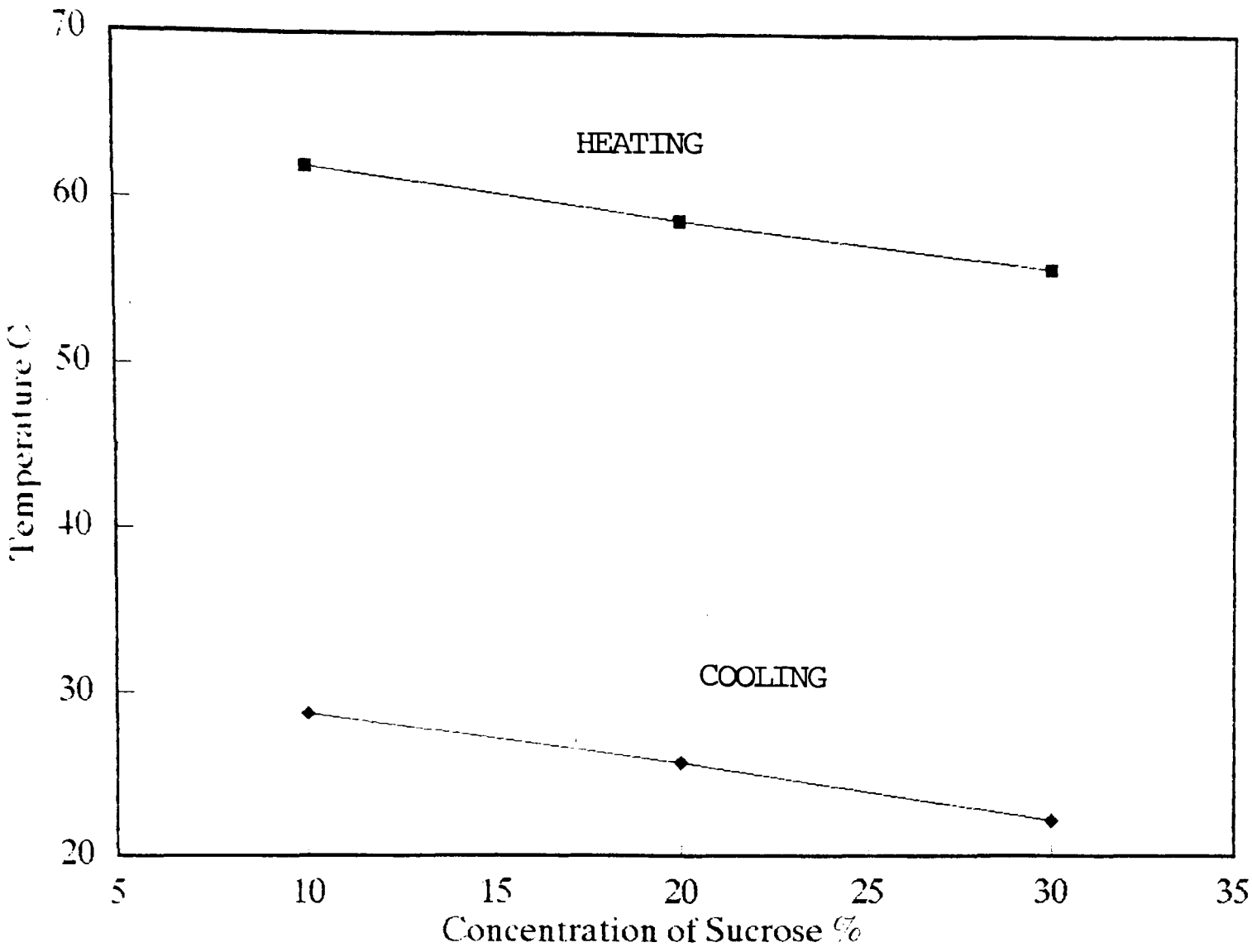


Figure 5.5 Transition midpoint temperatures for 0.75% A4M with varying concentrations of 50% sucrose, scan rate 0.5°/min.

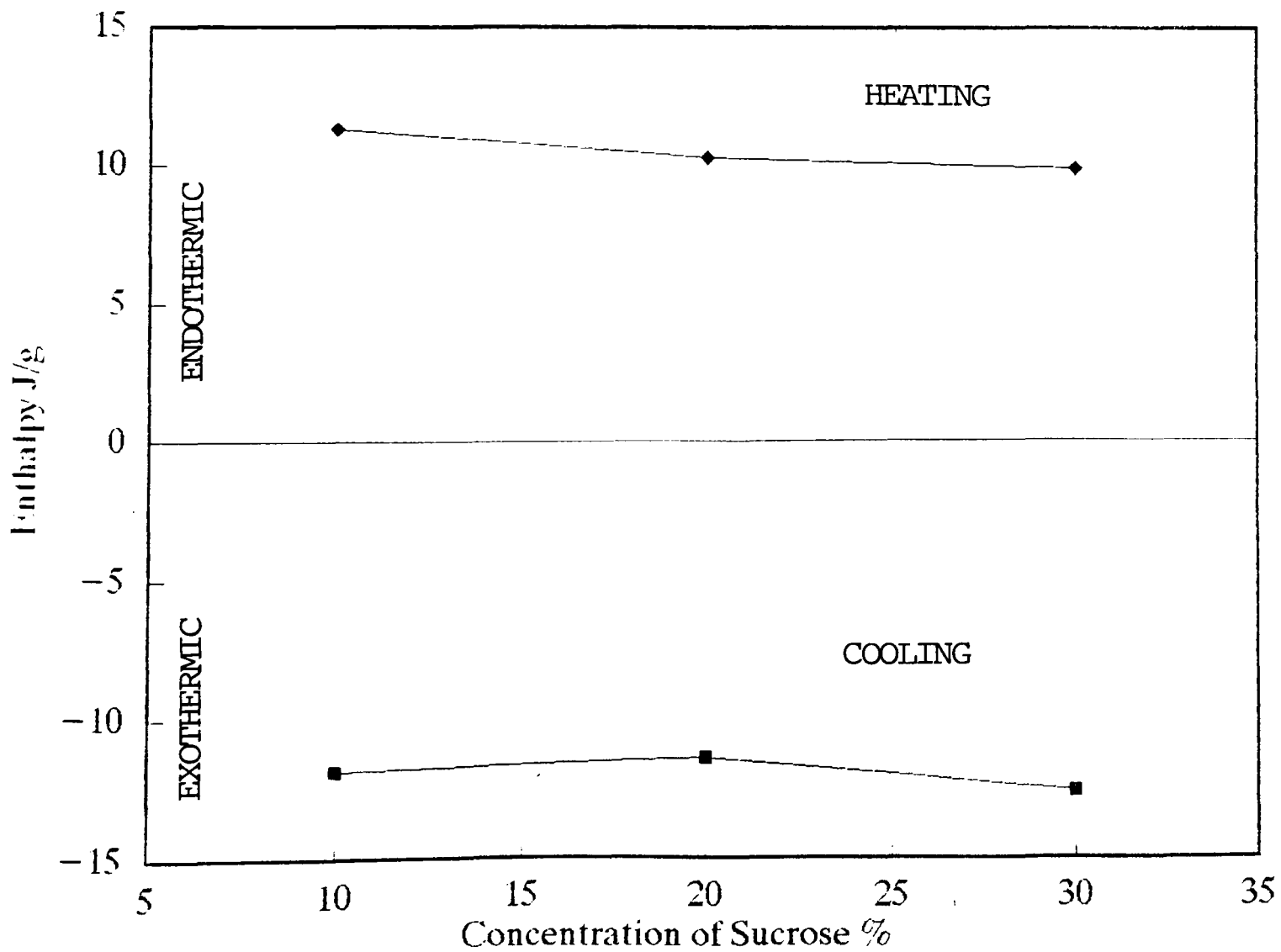


Figure 5.6 Transition enthalpies for 0.75% A4M with varying concentrations of 50% sucrose, scan rate 0.5°C/min.

5.3 Effect of Ethylene glycol on methylcellulose

5.3.1 DSC Investigations

DSC is used in this study to detail the thermally induced changes in conformation to provide an insight into the nature of the underlying molecular state changes of methylcellulose with modification of the solvent by the addition of ethylene glycol.

DSC scans were carried out at three different scan rates (0.2, 0.5 and 0.8 degrees per minute) for solutions of methylcellulose (A4M) with concentrations of ethylene glycol ranging from 0 to 20% w/w. Each sample was subjected to a heating regime from 5°C to 95°C, followed by cooling back to 5°C. The enthalpy was calculated automatically and plotted against the temperature. Individual scans are shown in the Appendix, but the significant changes in behaviour are summarised here.

Methylcellulose undergoes an endothermic transition on heating and an exothermic conformational change on cooling in the presence of ethylene glycol. The transitions are similar to the thermal changes observed in Section 5.2 with sucrose, with a single sharp peak for gelation during heating, and a double peak offset to lower temperatures in the cooling direction. Figure 5.7 shows that the enthalpy changes on gelation and dissociation are unaffected by ethylene glycol up to a concentration of ~ 10% w/w, but then decreases, with no detectable transitions by 20% w/w. The variation of transition midpoint temperature with scan rate, both on heating and on cooling, is slight (Figure 5.8), indicating that it arises solely from thermal lag due to finite rates of heat transfer within the calorimeter. Over the range of ethylene glycol concentrations at which thermal transitions could be detected (0 to 19% w/w) the position of the heating endotherm stays the same (Figure 5.9), but the cooling exotherm moves to progressively lower temperature. This is illustrated clearly in Figure 5.10 for methylcellulose in water, and with the addition of 17% w/w ethylene glycol. The

reduction in enthalpy change is also clearly evident, but there is no detectable change in co-operativity of the transition with increasing ethylene glycol (as measured by peak width).

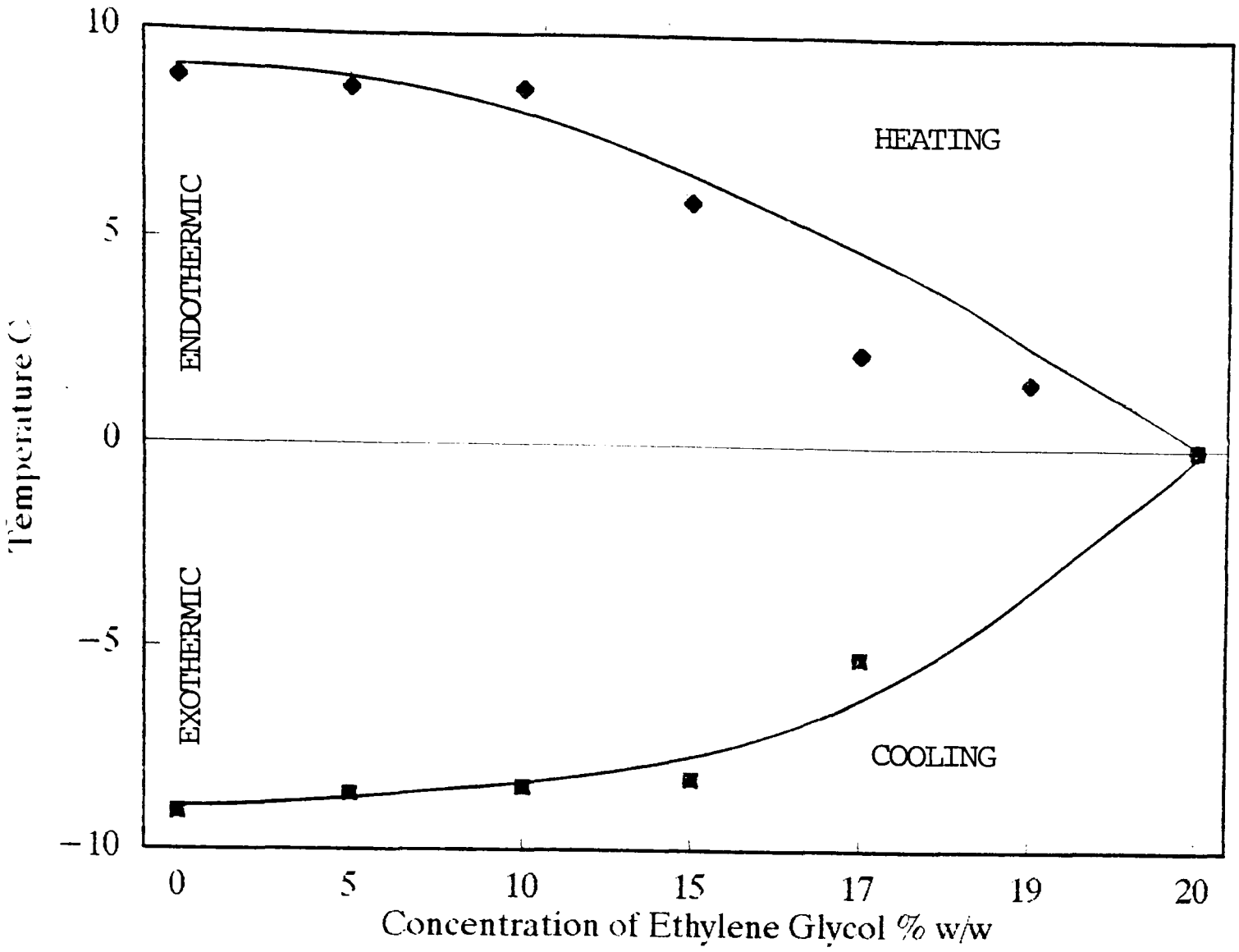


Figure 5.7 Transition enthalpies for 0.75% A4M with varying concentrations of ethylene glycol, scan rate 0.5°C/min.

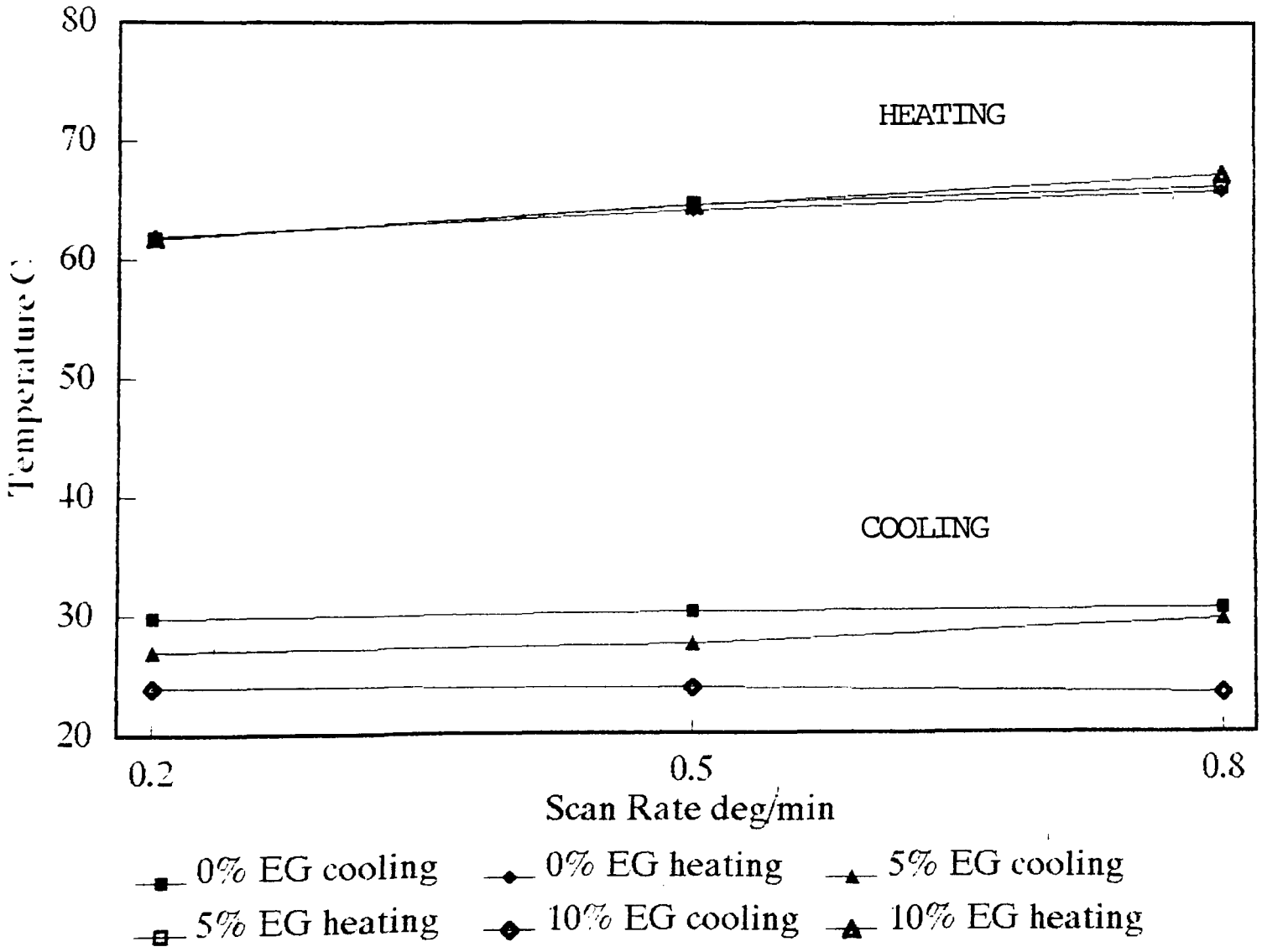


Figure 5.8 Transition midpoint temperatures of 0.75% A4M with varying scan rate and ethylene glycol concentrations.

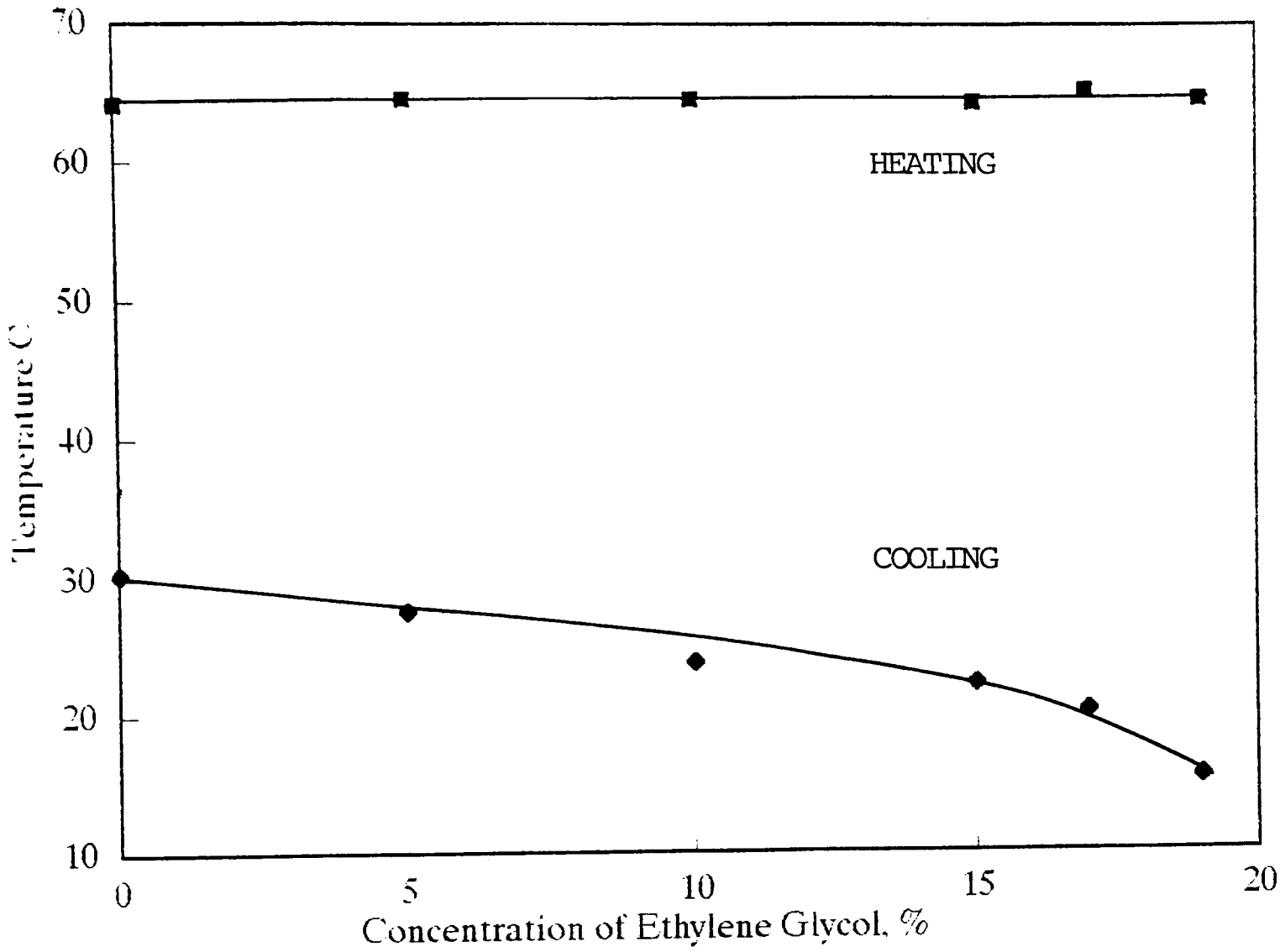
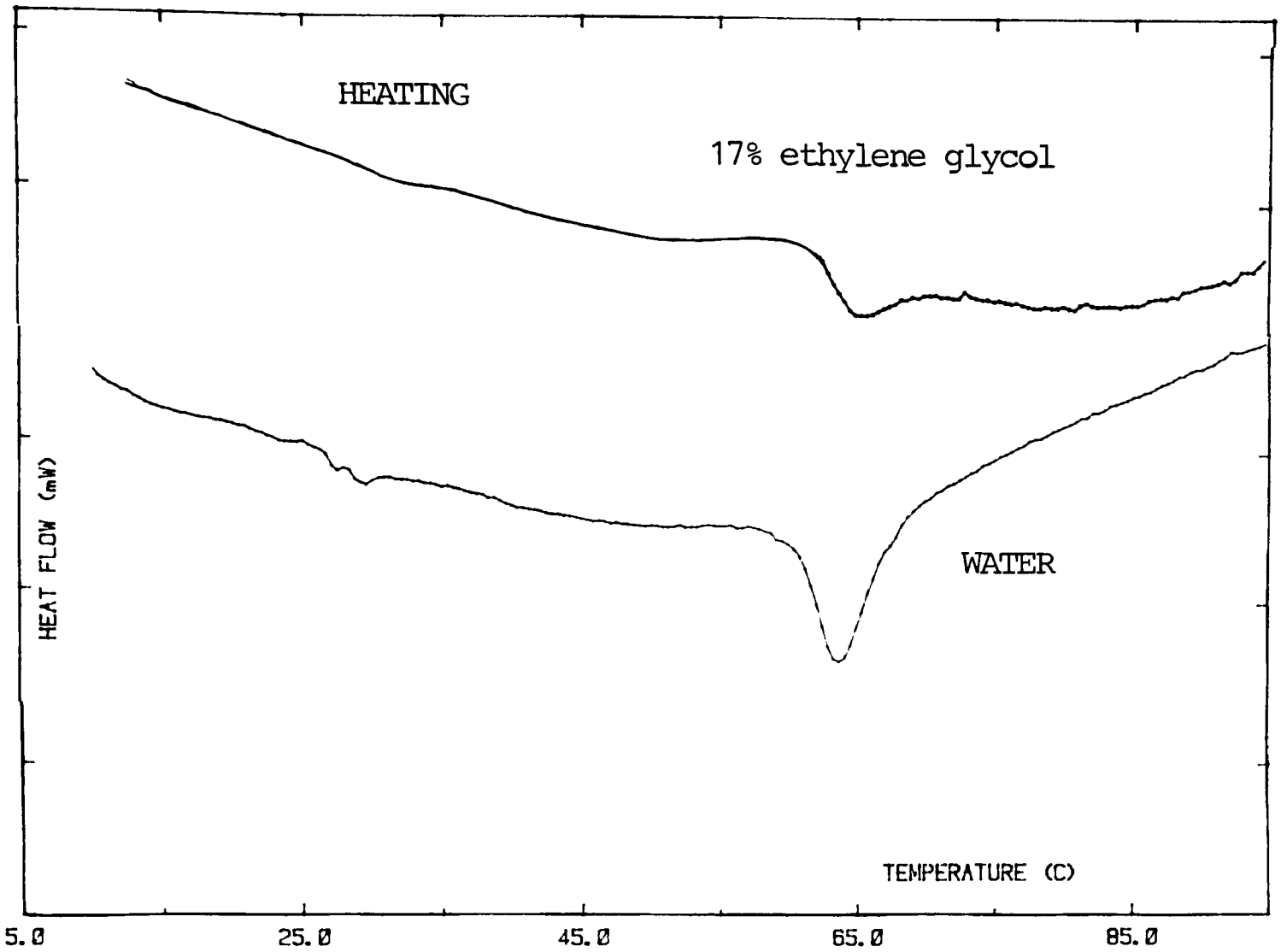


Figure 5.9 Transition midpoint temperatures for 0.75% A4M with varying concentration of ethylene glycol, scan rate 0.5°C/min.



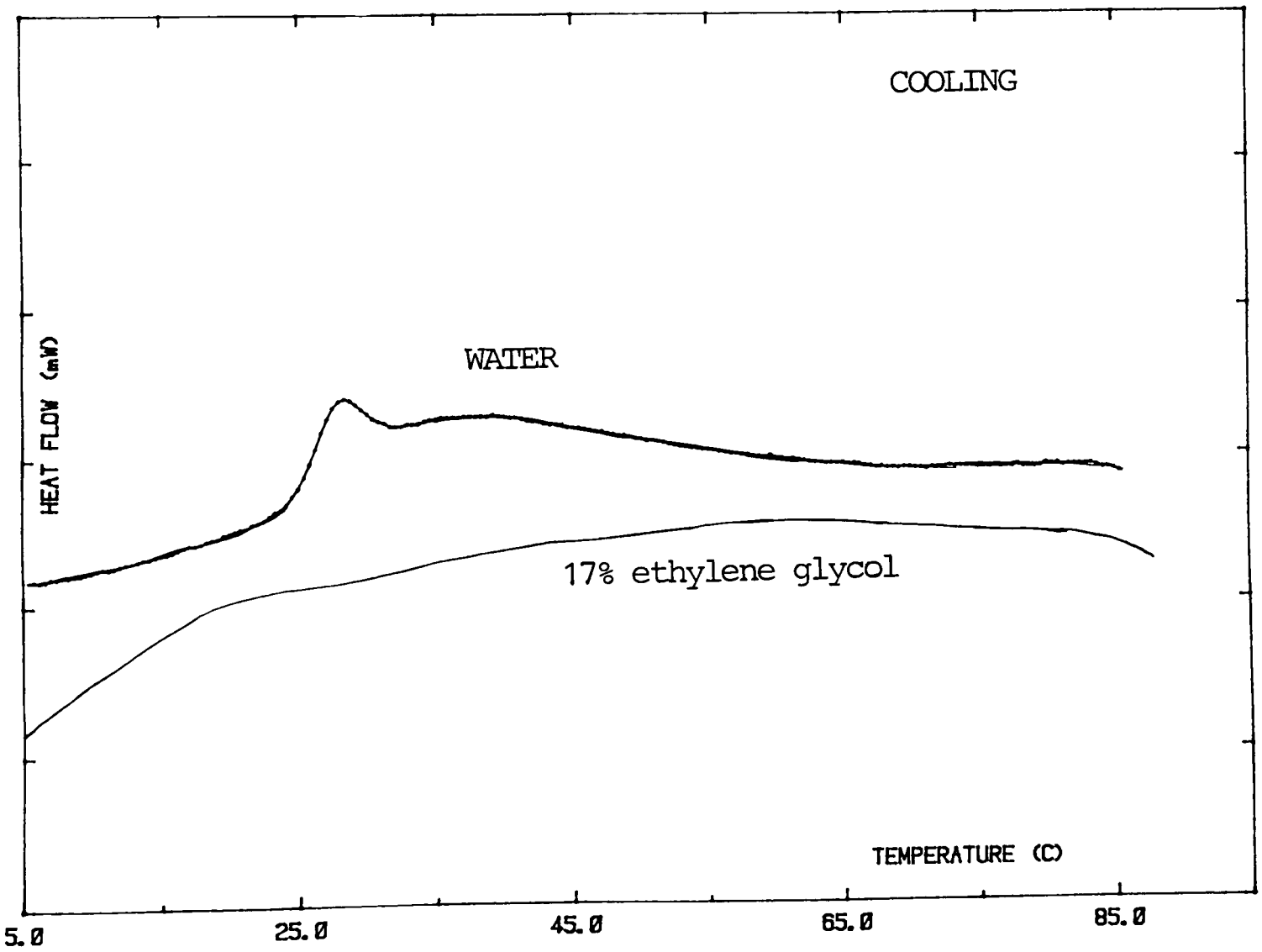


Figure 5.10 DSC scans on heating and cooling for 0.75% A4M in water and in 17% ethylene glycol, scan rate 0.5°C/min.

5.3.2 Mechanical Spectroscopy

Mechanical spectroscopy provides a sensitive measure of the molecular state changes involved during gelation and melting. The thermally induced transitions observed by DSC (Section 5.3.1) can be directly related to the formation and dissociation of the gel network by a measure of the structural moduli during heating and cooling. Modification of the solvent by the addition of ethylene glycol has shown specific changes in the enthalpic requirements of thermogelation, accompanying changes in the structural behaviour are expected.

The samples were prepared in exactly the same way as for DSC from a 3% w/w stock solution of methylcellulose, A4M, (Section 3.2) and were rigorously de-gassed before loading onto the pre-cooled plate of the rheometer. The samples were subjected to a heating regime from 5°C to 90°C, and subsequent cooling back to 5°C with an oscillation frequency of 1 rad/sec and a strain of 2%. Frequency sweeps were carried out at various temperatures during heating and cooling. The frequency was varied between 0.1 and 101 rad/sec at a strain of 2%.

As discussed in Section 1.4, hydrophobic gelation is normally considered to occur by "de-mixing" of polymer and solvent when the entropic advantages of disrupting 'cages' of structured water exceeds the enthalpic advantage of polymer-solvent interaction. As shown in Figure 5.11, however, thermogelation of methylcellulose does not occur as a single process as would be expected from a simplified overview of the "de-mixing" gelation process. Instead, there are two distinct 'waves' of increase in rigidity (G') observed on heating, with two corresponding 'waves' offset to lower temperature on cooling (roughly co-incident with the two thermal processes observed by DSC; Figure 5.1b). Similar behaviour has been observed previously (Haque *et al.*, 1993), and its possible origin is discussed below (Section 5.3.3).

The effect of increasing ethylene glycol concentration is illustrated in Figures 5.12 to 5.18, the involvement of two distinct processes on heating for methylcellulose in water (Figure 5.12) is also evident in the temperature-dependence of G" (the viscous modulus) and tan δ (where δ is the 'phase lag'). The changes in G' (the rigidity modulus) alone, on heating and cooling, are summarised in Figures 5.19 and 5.20 respectively. Addition of ethylene glycol has no systematic effect on the second 'wave' increase in G', but progressively eliminates the first 'wave' of structure formation by raising the gel-like character of the solution state at low temperatures toward that attained in water on completion of 'wave 1', but before the onset of 'wave 2'. The increase in G' at low temperature (prior to gelation on heating) with increasing concentration of ethylene glycol is illustrated in Figure 5.21. Ethylene glycol concentration, however, has no systematic effect on gel rigidity at high temperatures (Figures 5.19 and 5.20), with G' values of the same order of magnitude.

There is little change in the temperature for the onset of the second (major) gelation process during heating (Figure 5.22), but the temperature at which the gel structure begins to dissociate on cooling decreases steeply with increasing concentration of ethylene glycol, with gels above 40% w/w remaining intact at 0°C (as indicated both by the instrumental values of G' shown in Figure 5.20 and by visual inspection). Figures 5.23 to 5.25 show the mechanical spectra (frequency-dependence of G', G" and the dynamic viscosity; η*) obtained at selected temperatures on heating and cooling for 0.75% w/v A4M in the presence of, respectively, 5, 20 and 40% w/w ethylene glycol. The progressive retention of gel-like character on cooling is again evident, with the sample in 40% ethylene glycol showing the response typical of a strong gel at the lowest temperature at which measurements could be made (~ 6°C).

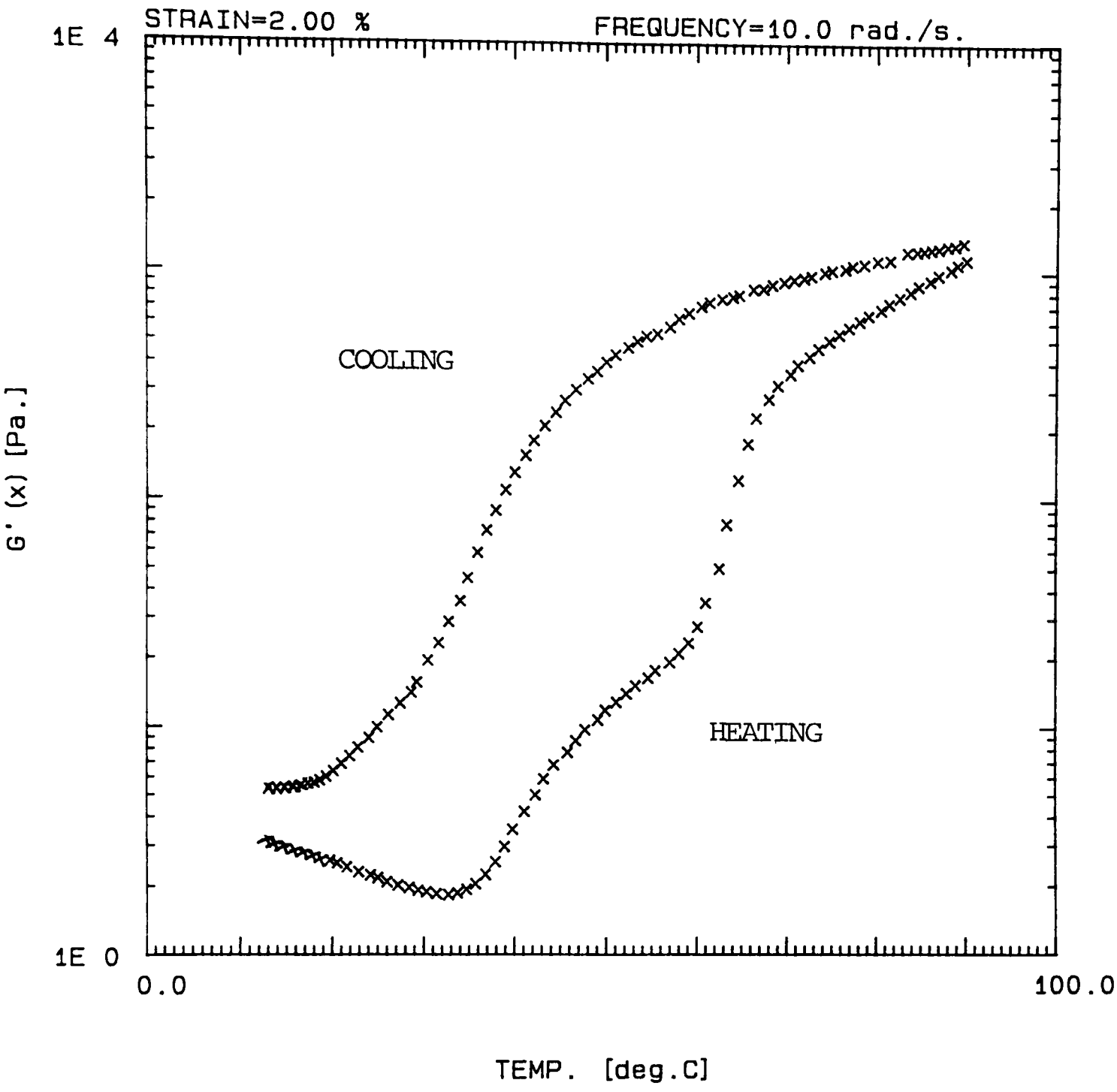
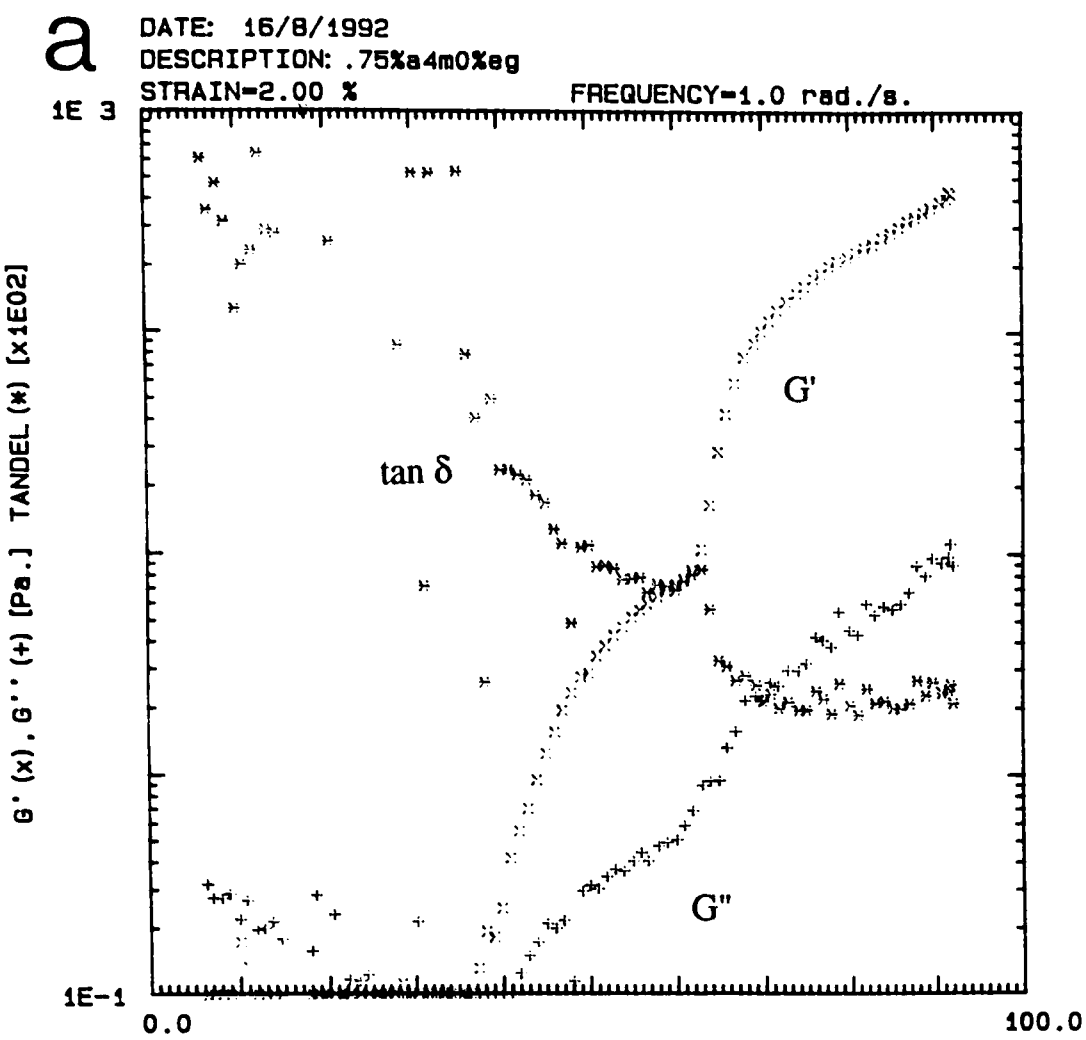


Figure 5.11 Changes in G' on heating and cooling at 1°C/min for 0.75% A4M in water.



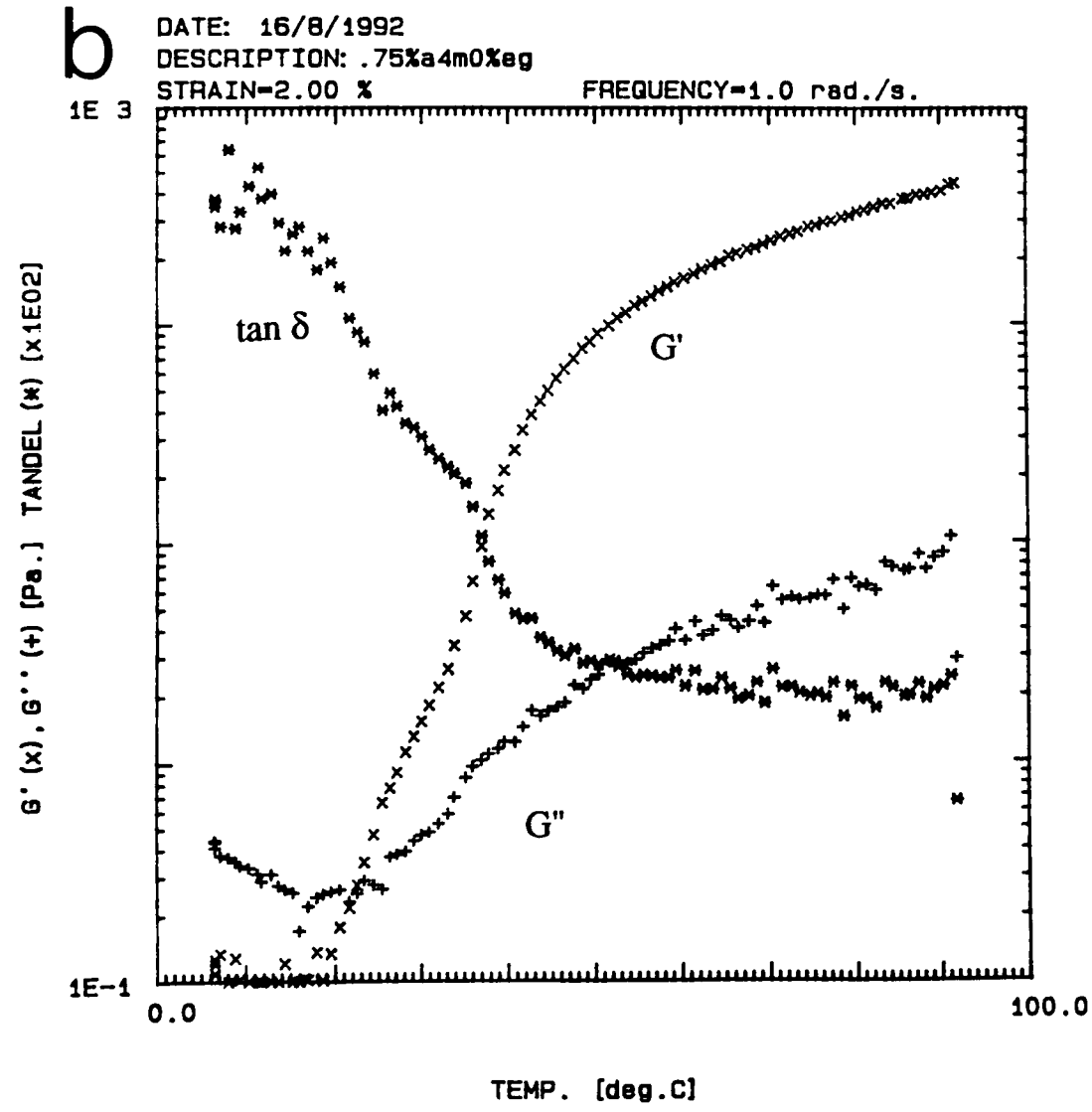


Figure 5.12 Changes in structural moduli of 0.75% A4M in water, a) heating, b) cooling, scan rate 1°/min.

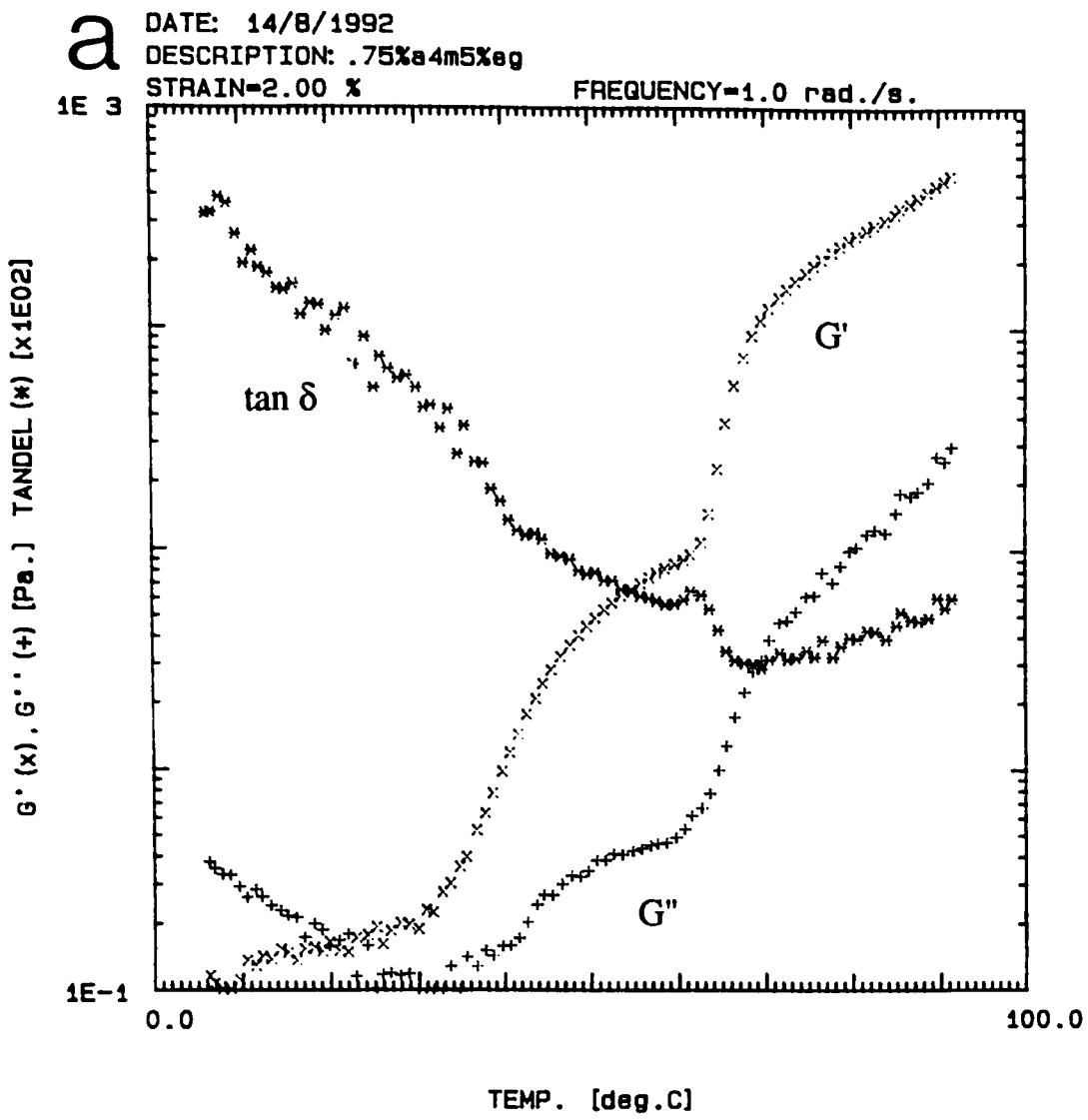


Figure 5.13 Changes in structural moduli of 0.75% A4M with 5% ethylene glycol a) heating, b) cooling, scan rate 1°/min.

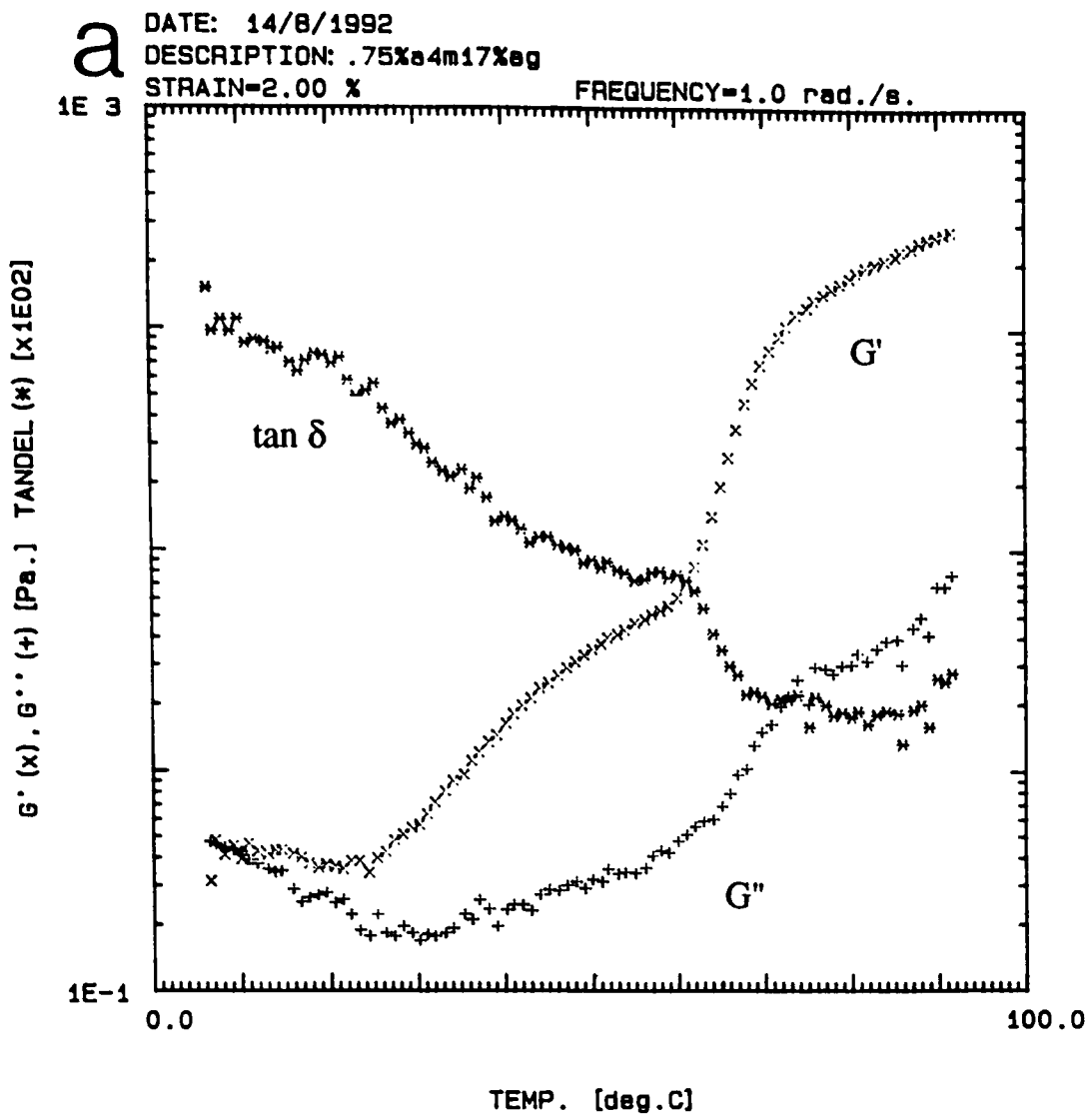
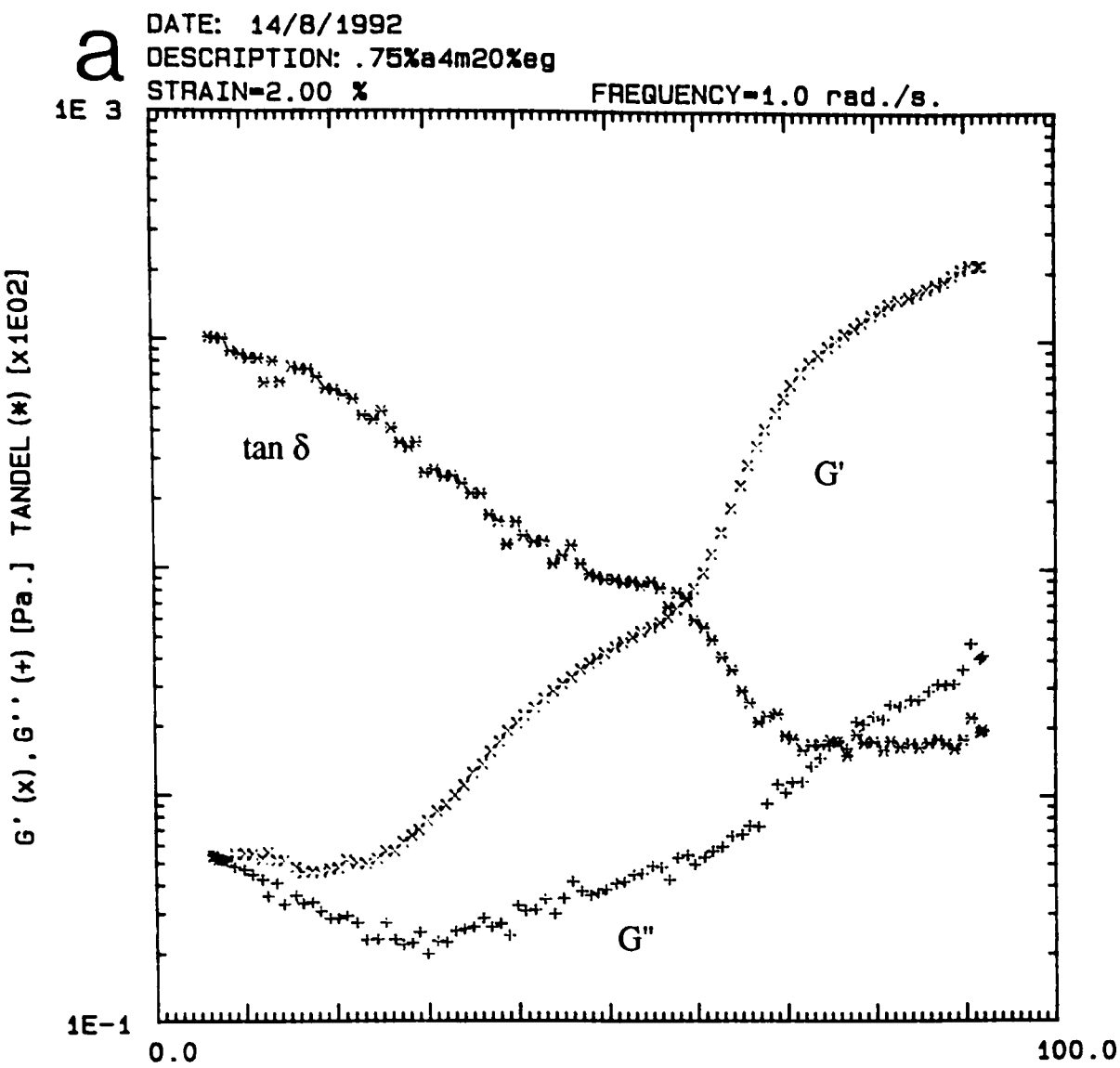


Figure 5.14 Changes in structural moduli of 0.75% A4M with 17% ethylene glycol a) heating, b) cooling, scan rate 1°/min.



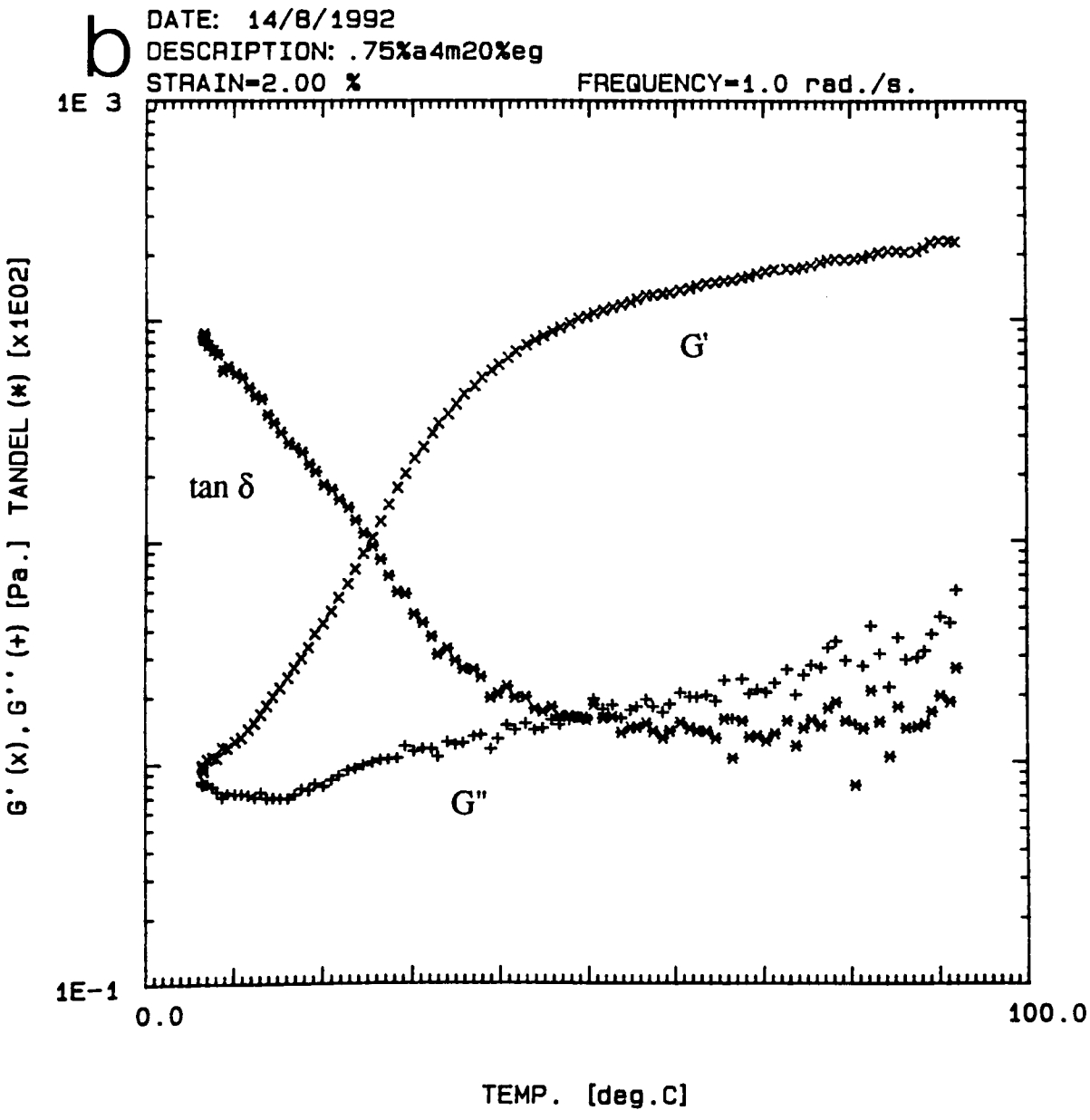


Figure 5.15 Changes in structural moduli of 0.75% A4M with 20% ethylene glycol a) heating, b) cooling, scan rate 1°/min.

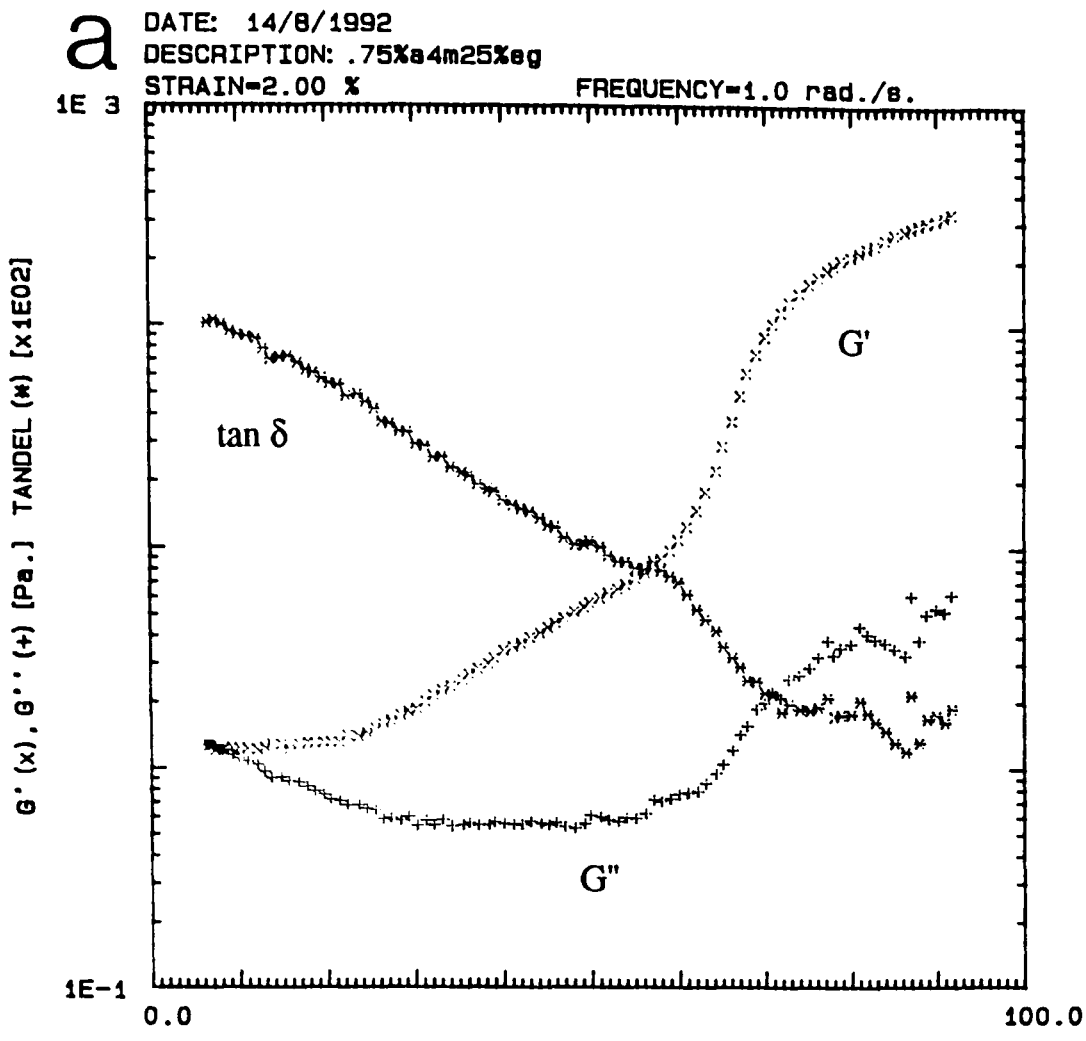
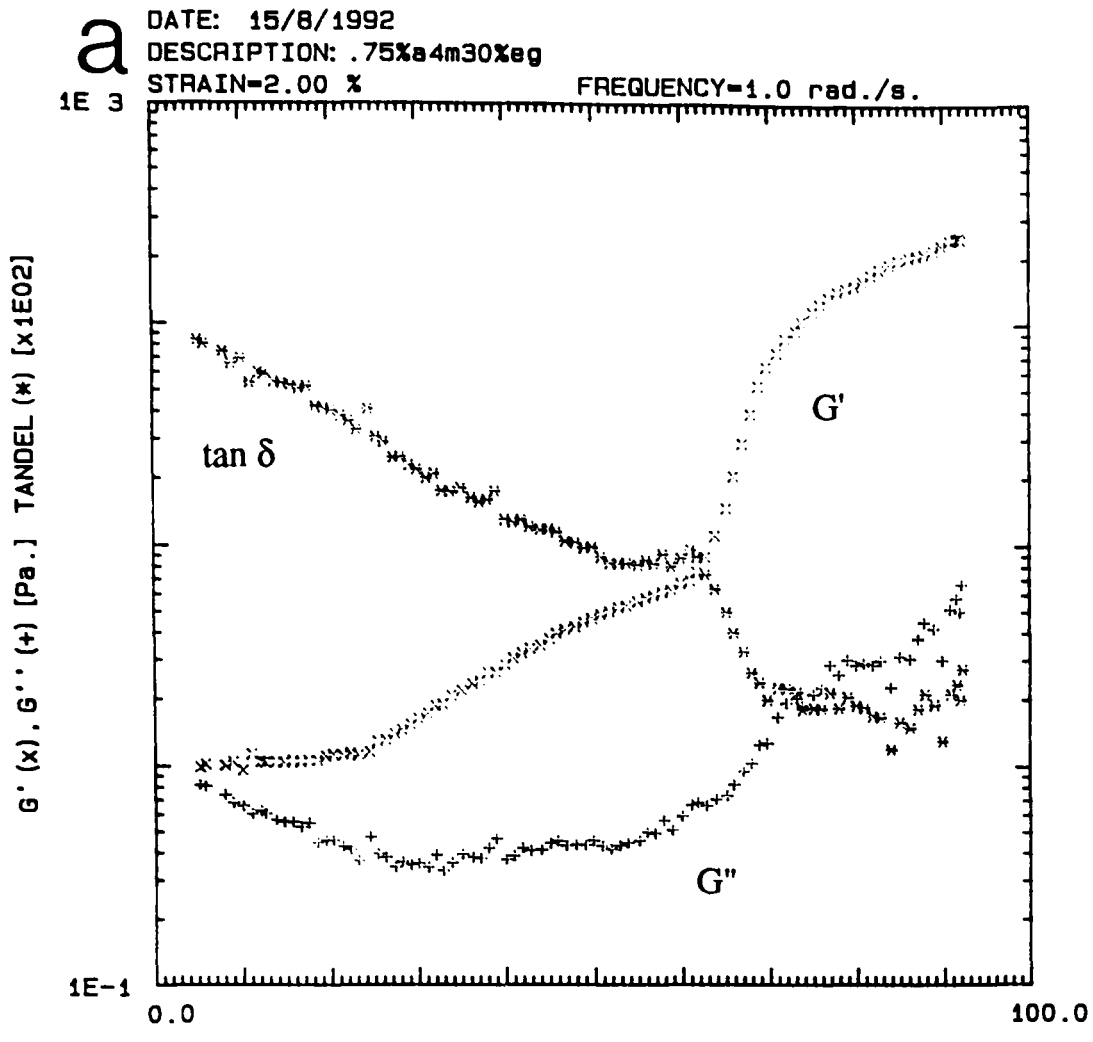


Figure 5.16 Changes in structural moduli of 0.75% A4M with 25% ethylene glycol a) heating, b) cooling, scan rate 1°/min.



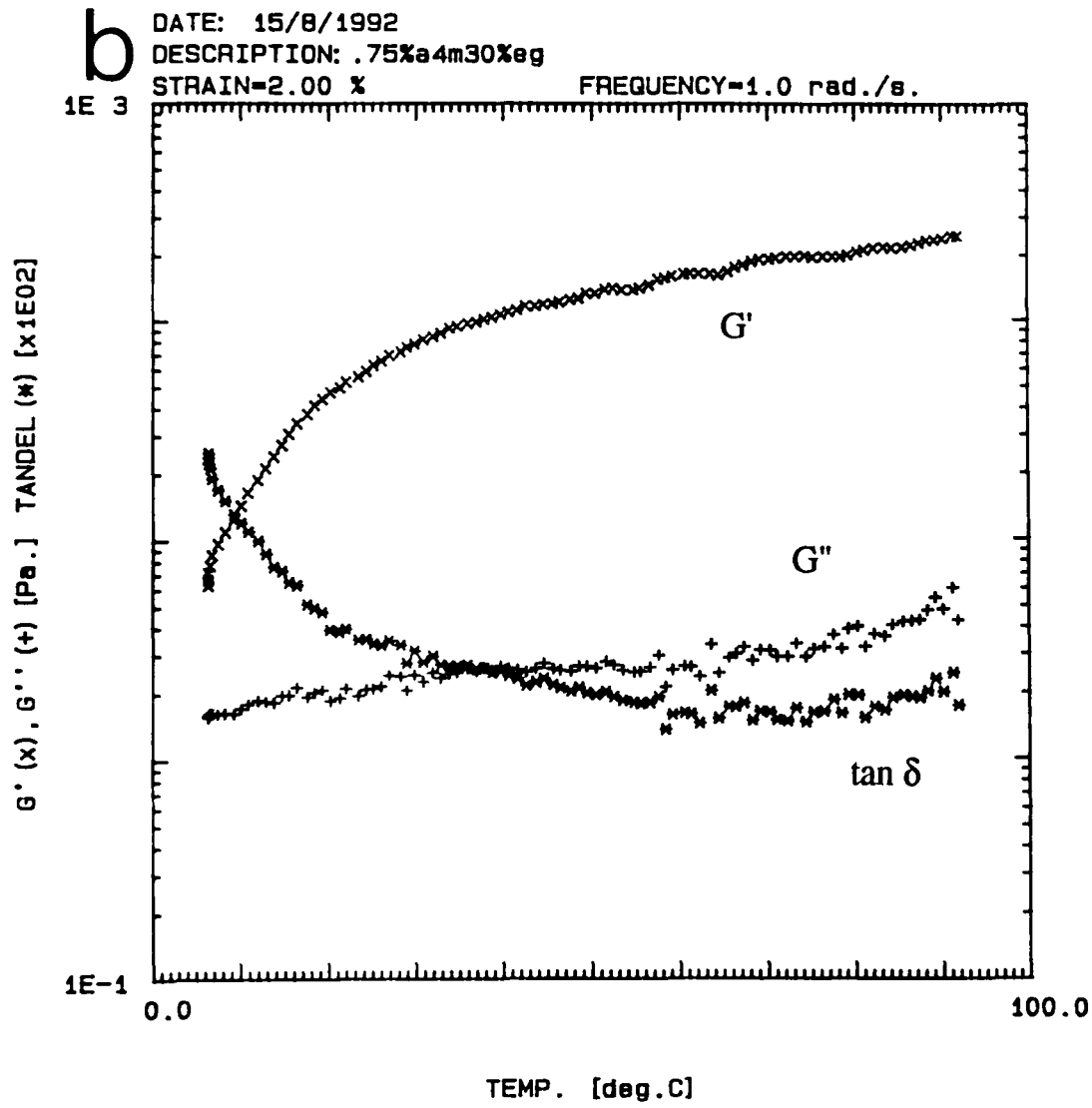


Figure 5.17 Changes in structural moduli of 0.75% A4M with 30% ethylene glycol a) heating, b) cooling, scan rate 1°/min.

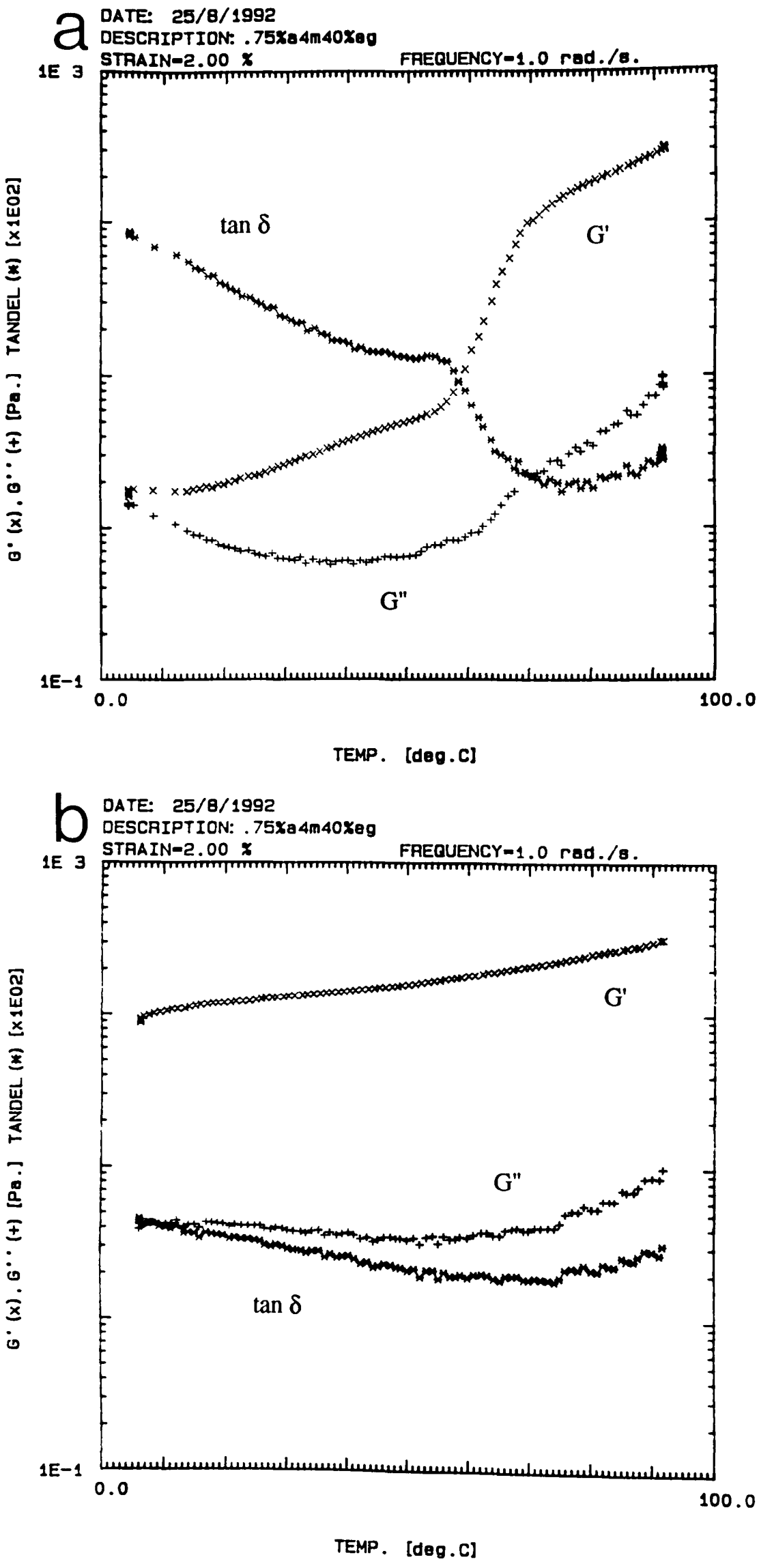


Figure 5.18 Changes in structural moduli of 0.75% A4M with 40% ethylene glycol a) heating, b) cooling, scan rate 1°/min.

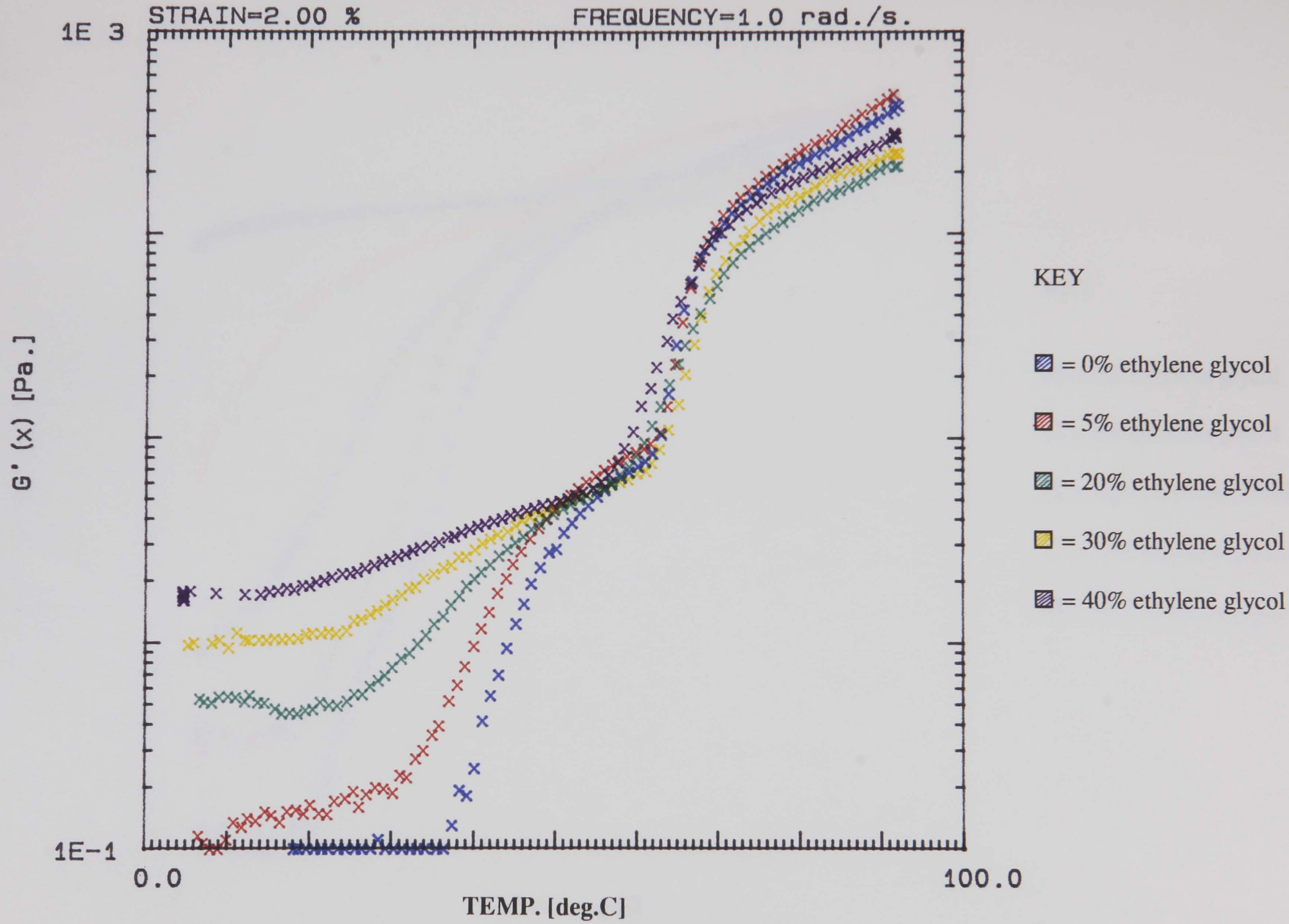


Figure 5.19 Changes in G' of 0.75% A4M on heating with increasing ethylene glycol concentration

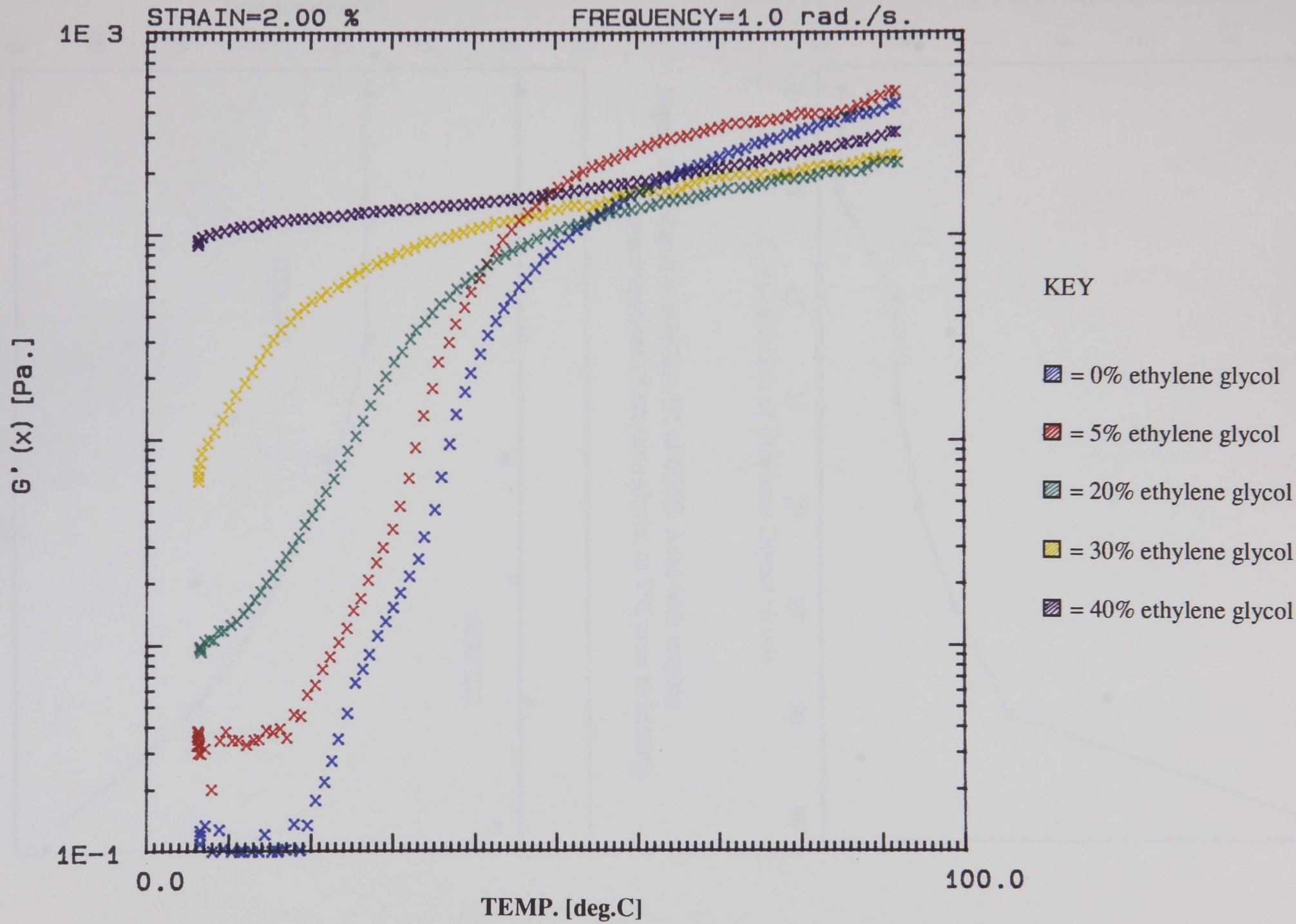


Figure 5.20 Changes in G' of 0.75% A4M on cooling with increasing ethylene glycol concentration.

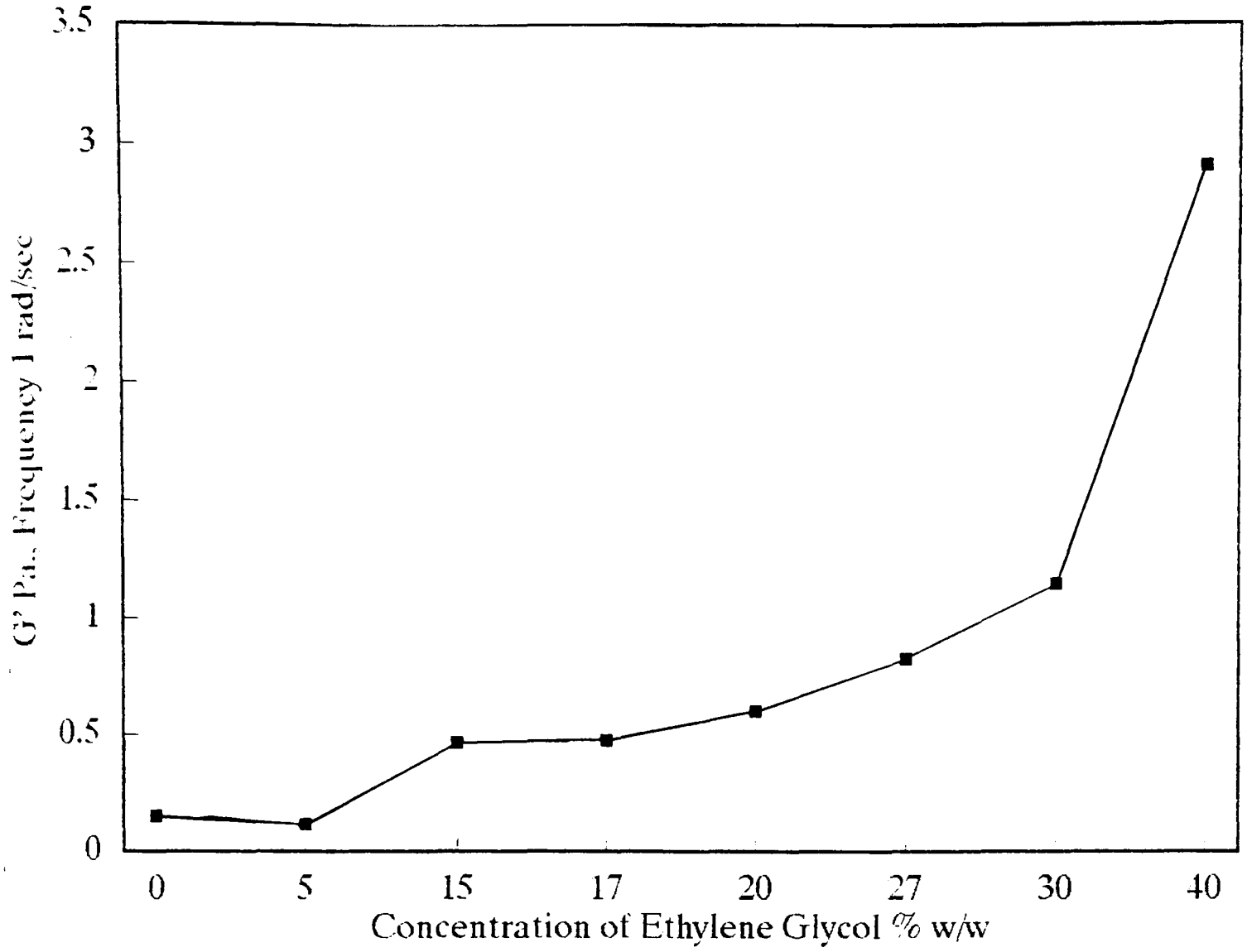


Figure 5.21 Rigidity modulus, G', of 0.75% A4M with varying concentrations of ethylene glycol, at 5°C prior to heating.

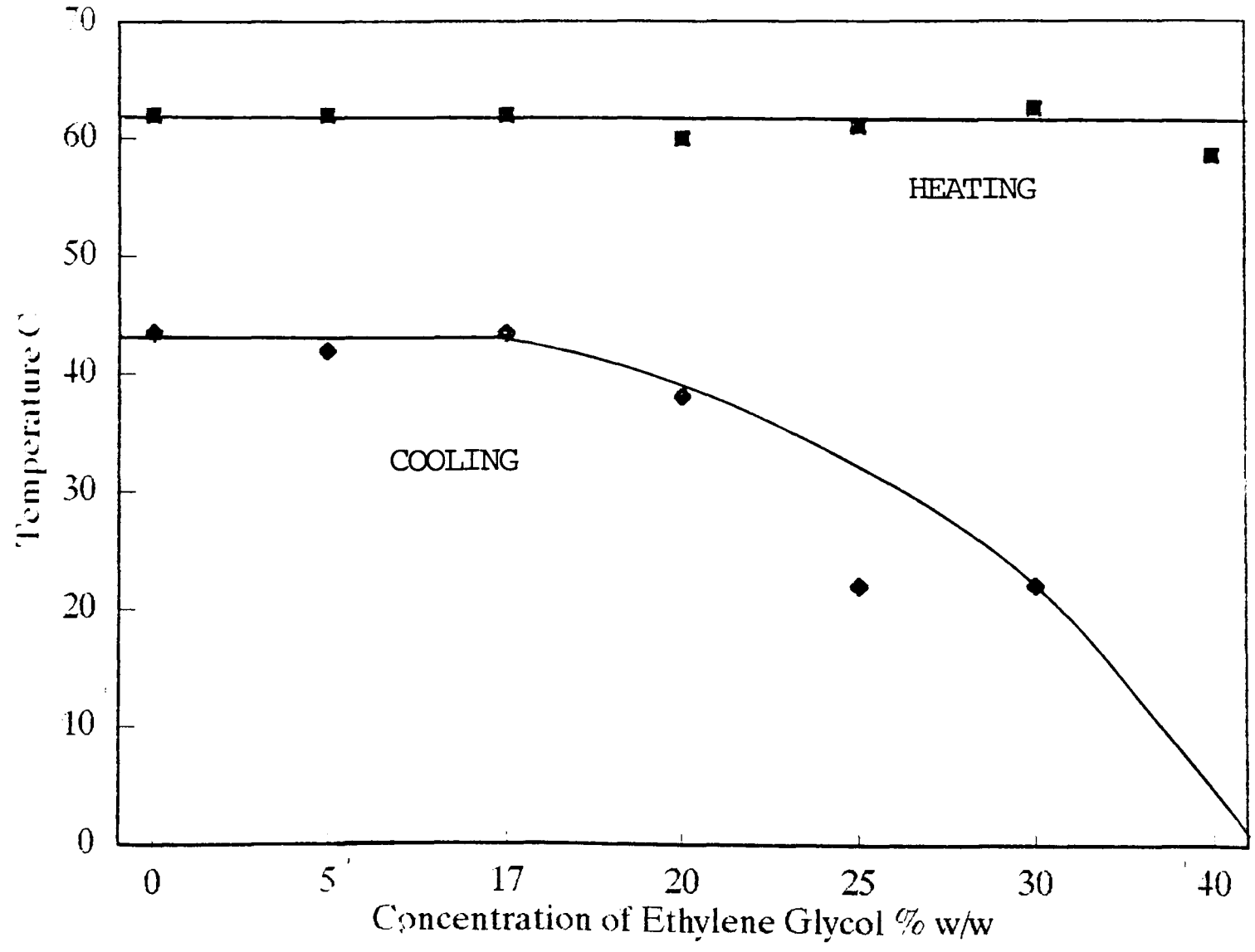


Figure 5.22 Temperature for the onset of thermal transitions of 0.75% A4M with varying concentrations of ethylene glycol.

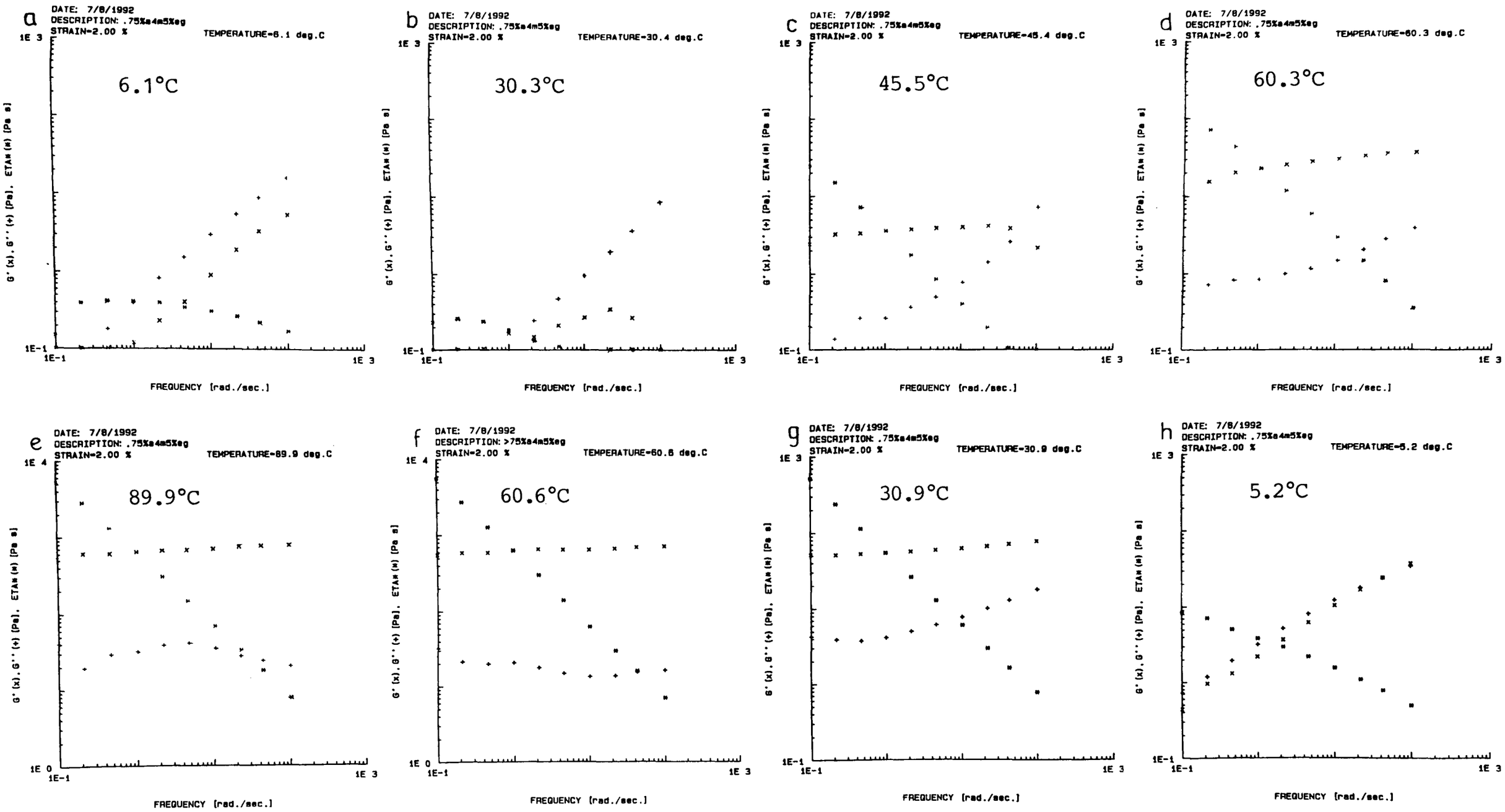


Figure 5.23 Mechanical spectra for 0.75% A4M, 5% ethylene glycol, heating a to e, cooling f to h.

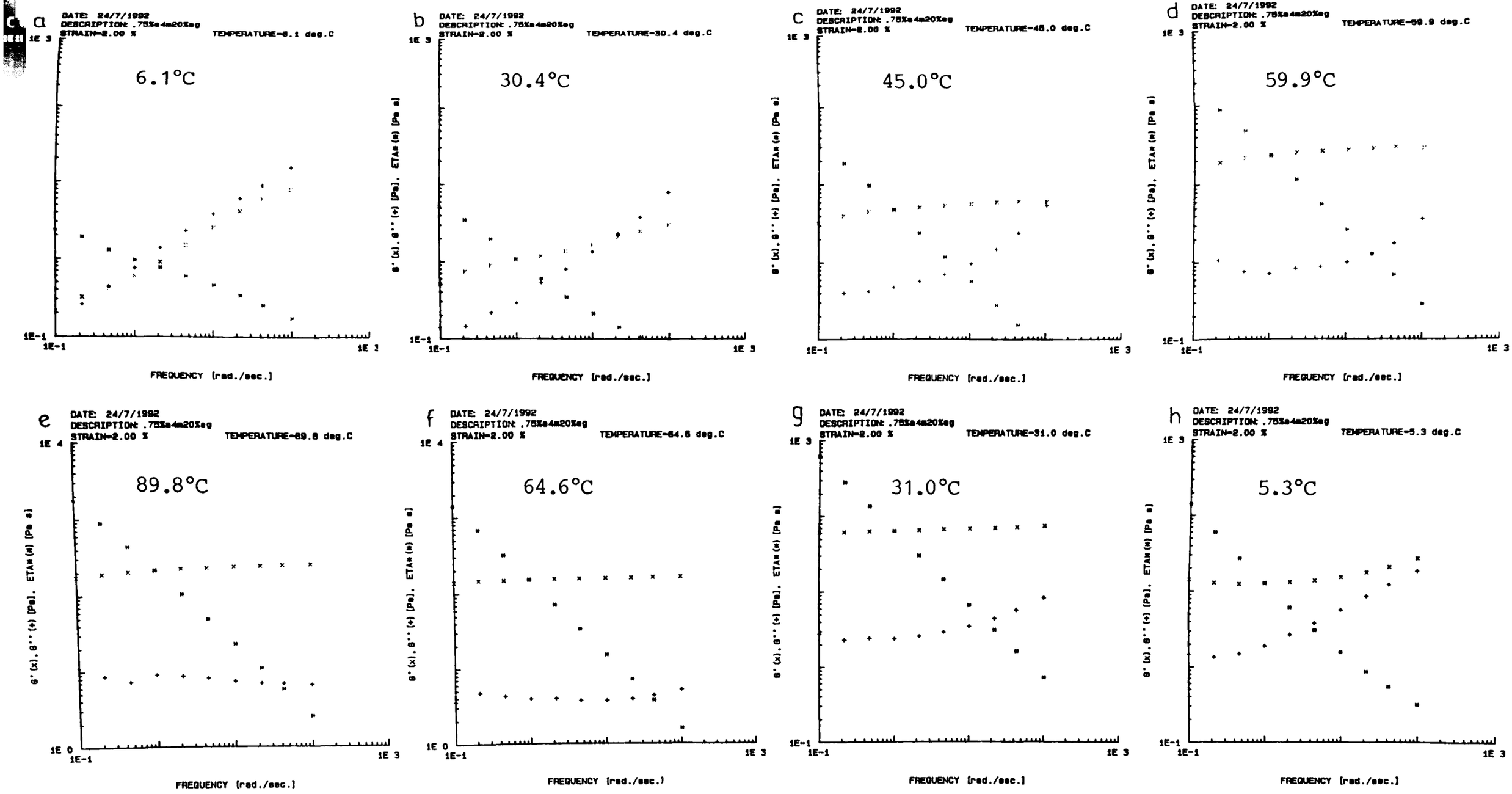


Figure 5.24 Mechanical spectra for 0.75% A4M, 20% ethylene glycol, heating a to e, cooling f to h.

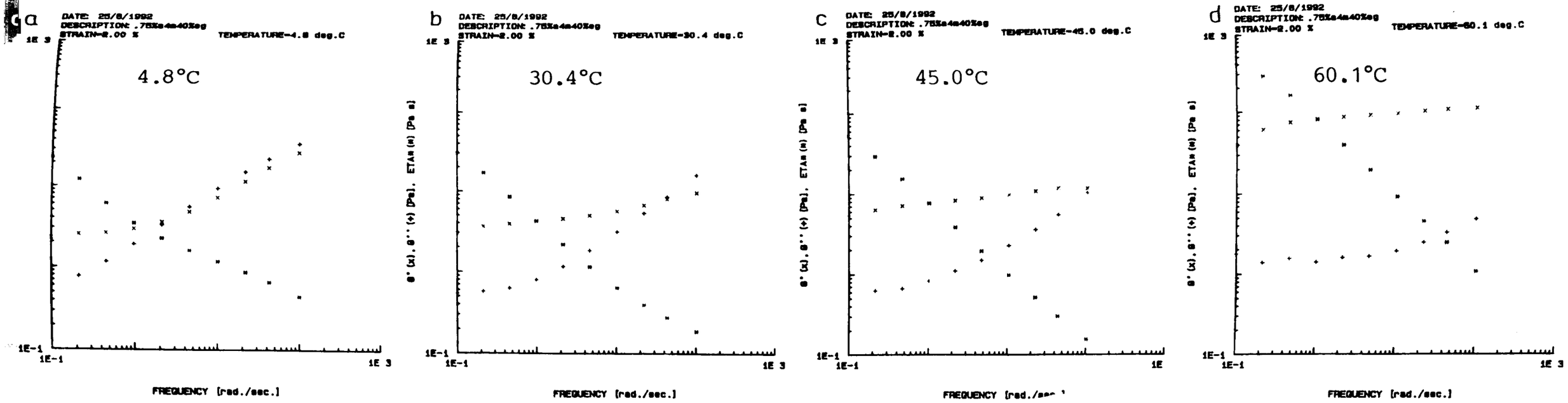


Figure 5.25 Mechanical spectra for 0.75% A4M, 40% ethylene glycol, heating a to e, cooling f to h.

5.3.3 Interpretation

In their recent investigation of the thermal gelation of methylcellulose in water, Haque *et al.* (1993) found that the first 'wave' of increase in G' (Figure 5.11) was accompanied by an increase in the clarity of the sample (% transmission of light) and also by an increase in the optical rotation (Figure 5.26) with thermal hysteresis similar to that observed for the formation and melting of aggregate structures seen for agar and κ-carrageenan (Dea *et al.*, 1972, Arnott *et al.*, 1974). There is also an associated increase in detectable ^{1}H NMR (Haque *et al.*, 1993) during the first stage increase in G', indicating an increase in conformational chain mobility. With a further increase in temperature the solution becomes turbid, behaviour associated with polymer-solvent "de-mixing" during gelation, and a coincident loss of detectable ^{1}H NMR as chain mobility is decreased during formation of the gel network. This evidence leads to the conclusion that some form of aggregate structure is 'melting-out' during the initial stages of heating prior to gelation. Haque *et al.* (1993) concluded that there was some form of cellulosic 'bundle' structure present in solution with unsubstituted regions of native cellulose linking the chains together.

In order for gelation to take place the associating hydrophobic groups must be brought into contact with each other, and melting of the postulated cellulosic 'bundles' is seen as a prerequisite for exposure of the methyl groups prior to hydrophobic interaction. Gelation proceeds as discussed in Section 1.4, with the formation of water 'cages' which then respond to heating to give hydrophobic intermolecular associations. This is directly analogous to the unfolding of native globular proteins to expose the hydrophobic regions of the protein core preceding gelation. The purpose of this study is to explore the validity of this theory by modification of the solvent by ethylene glycol.

From Figure 5.21, it is seen that there is an increase in G' at low temperature with increasing ethylene glycol concentration. This response of the polymer system to the modification of solvent quality can be interpreted in a similar manner to that indicated for pectin in Section

4.6. Ethylene glycol will be a better solvent for the hydrophobic, methyl groups, leading to a progressive solubilisation of the bundles, hence, the increase in G' at low temperatures, the more ethylene glycol present the greater the degree of solubilisation. This is analogous to the swelling of starch granules during gelatinisation to form a viscoelastic paste, but brought about by changes in the solvent quality. Ethylene glycol will have no effect upon the unsubstituted regions of the methylcellulose polymer and these unmodified sequences remain associated at all concentrations.

The loss in reversibility of the gelation process, following the interpretation offered by Haque *et al.* (1993), can also be attributed to the progressive destabilisation of the native 'bundle' structure. Formation of the gel is seen to be entropically, not enthalpically driven, as explained in Section 1.4. The solution state is enthalpically more stable than the gel network, however, the solubilised cellulose nucleates are less thermodynamically stable than the initial bundles formed in water alone. Because reformation of the fibrillar structure causes a loss in entropy, gel dissociation is a balance between the enthalpic advantage of the 'bundle' structure over the entropic drive to maintain the disordered gel network. On cooling, displacement of the dissociation of the gels to progressively lower temperatures (with 40% w/w ethylene glycol gel remaining intact) is explained by a decrease in the thermodynamic advantage of reforming bundles.

The loss of the DSC peaks at high concentrations of ethylene glycol can be explained by the origin of the peaks in water having a greater enthalpic advantage from "hydrocarbon-water" interactions as opposed to "hydrocarbon-hydrocarbon" interactions (Tanford, 1980). The formation of ordered 'cages' of water surrounding the methyl groups is enthalpically favourable and dominates over the loss in entropy incurred. As exposed methyl groups in the intermediate solution state (between 'waves' 1 and 2) become surrounded by ethylene glycol solvent rather than by water, the enthalpic advantage is lost. The system progressively resembles "hydrocarbon-hydrocarbon" interaction in both the intermediate and gel states involving no enthalpic transition and giving rise to the initial increase in G' at lower temperature.

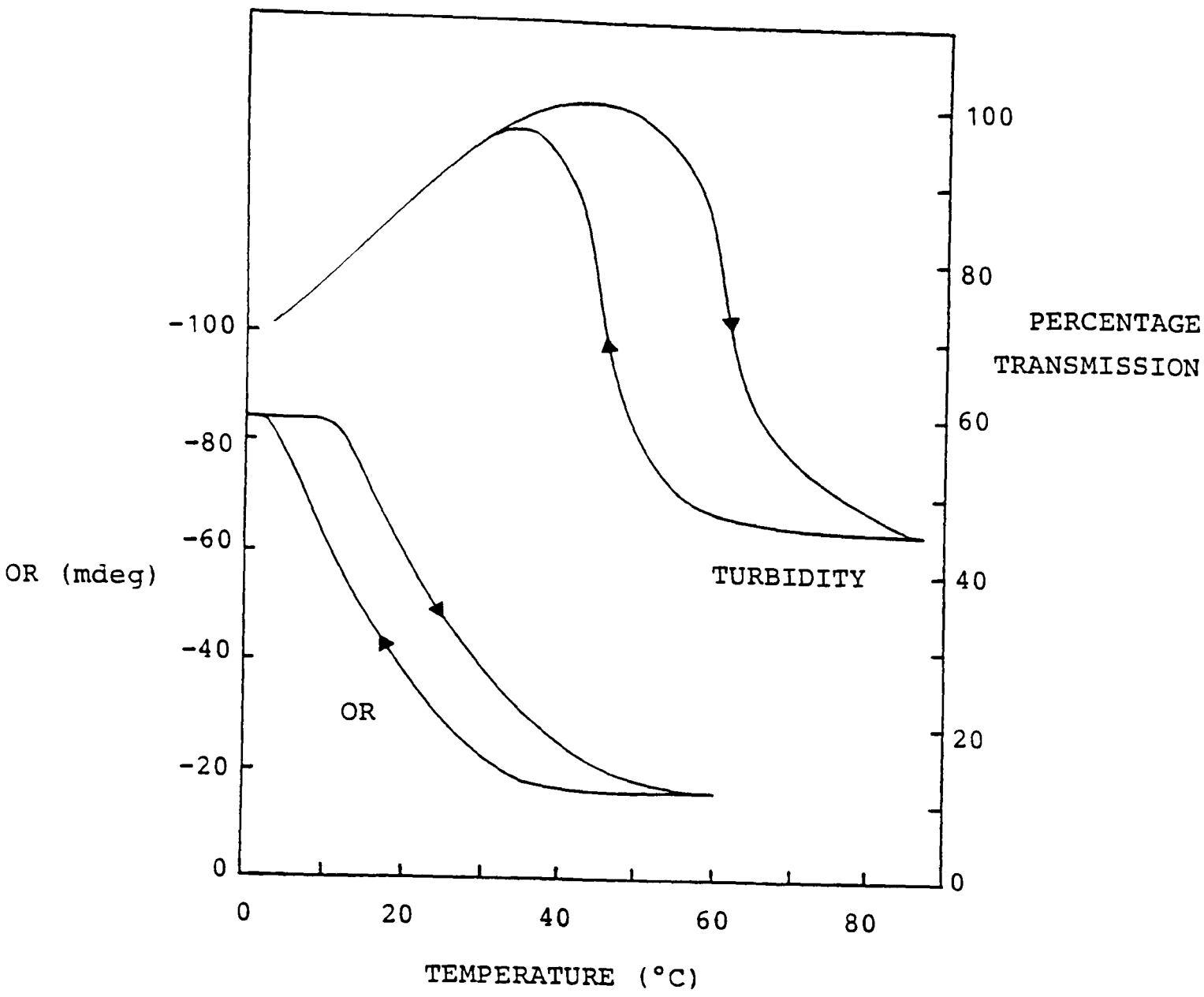


Figure 5.26 Changes in conformation and turbidity of methylcellulose (0.25% A4M) on heating and cooling as monitored, respectively, by optical rotation (10 cm pathlength; 365 nm; Perkin-Elmer 241 polarimeter) and percentage transmission (1 cm pathlength; 460 nm; Unicam SP 1800 spectrophotometer). (From Haque *et al.*, 1993).

5.4 Hydroxypropylmethylcellulose

In order to extend the argument a brief study was carried out to explore the effect of introducing hydroxypropyl substituents, containing a polar hydroxyl group, onto the cellulose chain.

The HPMC samples were prepared from a 3% stock solution (Section 3.2) and de-gassed thoroughly prior to loading onto the pre-cooled plate of the rheometer. The samples were subjected to oscillatory shear during a heating and cooling regime between 5°C and 90°C.

Figure 5.27 shows a comparison between the mechanical spectra of HPMC (K4M) and methylcellulose (A4M) in water, at 5°C prior to heating. It can be seen that they are virtually identical, indicating that there is no difference in solubility of the samples in water. Having introduced the concept of cellulosic bundles being present at low temperatures in methylcellulose (Section 5.3.3), we can suggest from this evidence that a similar 'bundle' structure must be present in HPMC at low temperature. However, the gel structure formed at high temperature (Figure 5.28) is much weaker in HPMC than methylcellulose. This is a well known phenomenon (Sarkar, 1979, Grover, 1986) and is used in industry as a route to manipulating the physical properties of the cellulose ether. The rationalisation of this weakening in gel strength with increasing hydroxypropyl substitution is attributed to a decrease in hydrophobicity of the polymer causing a reduction in the efficiency of promoting hydrophobic association.

Of greater relevance to the present investigation, however, is the response of the system to changes in the quality of the solvent by the addition of ethylene glycol. Full mechanical spectra obtained for heating and cooling with the addition of 10, 20, 25 and 35% w/w ethylene glycol, and in water alone, are reproduced in the Appendix. Figure 5.29 shows the change in G' during heating and cooling for HPMC in water and in 10% ethylene glycol. The detailed form of the temperature course of the modulus change cannot be directly compared

with the results shown in Figures 5.19 and 5.20 for methylcellulose, because the gel structure is much weaker it was not possible to obtain stable measurements at the same low frequency (1 rad/sec) and the response was only readily detectable at a higher frequency (10 rad/sec). However, in the context of the present argument, it is important to note that the recovery of the initial solution state at low temperature is complete for the system in water (Figure 5.29), whereas, in the presence of 10% ethylene glycol there is considerable retention of the gel-like structure. As shown in Figure 5.30, with 20, 25 and 35% w/w ethylene glycol, there is no evidence of a further systematic increase in the effect with higher concentrations (up to 35%). It would therefore appear, that when hydroxypropyl groups are present the postulated bundles are destabilised more readily than in the presence of methyl substitution alone. Addition of only 10% ethylene glycol is sufficient to cause destabilisation of the fibrillar structure to the extent where gel network is almost completely retained (due to the loss of the thermodynamic advantage for gel dissociation), whereas, with methylcellulose, there was a progressive build-up to 40% ethylene glycol with no loss of G' on cooling.

From this evidence we can conclude that the presence of hydroxypropyl substituents renders the cellulosic bundles far more sensitive to modification of the solvent by ethylene glycol and that they dissociate more readily. There are three main reasons as to why this should be so. Firstly, there is a large difference in molecular size of the hydroxypropyl substituent in comparison with the methyl substituent, this will lead to a degree of steric hindrance involved in the packing of the cellulosic bundles in the presence of the hydroxypropyl groups. Secondly, packing of the hydroxypropyl group, with internal bonding, into an ordered structure will cause a loss of conformational entropy due to a reduction in the degree of rotational freedom available about the carbon-carbon bonds. And thirdly, each hydroxypropyl substituent contains a polar hydroxyl group (capable of forming hydrogen bonds with the water) which renders the polymer slightly more hydrophilic and antagonises the hydrophobic effect of the methyl groups in hydrophobic clustering. All of these factors lead to an incipient destabilisation of the cellulosic bundle structure at low temperature and a reduction in the enthalpic drive for reformation of the solution state subsequent to gelation, resulting in hysteresis and retention of G' as discussed above.

5.5 Summary

The overall conclusions which can be drawn from this work, are that the behaviour of amphiphilic polysaccharides depends both on the balance of hydrophobic and hydrophilic character within the polymer, and also on the ability of the solvent to 'dissolve' one or other, or both, of the hydrophobic and hydrophilic components.

It appears for the systems studied here, that the combination of water and ethylene glycol as a solvent is effective at 'dissolving' both polymer types, shown by the loss of gelation of very high methoxy pectin with greater than 60% ethylene glycol, and the loss of the 'bundle' structure in both methylcellulose and hydroxypropylmethylcellulose resulting in retention of the gel structure on cooling. However, the ratio of hydrophobic to hydrophilic components of the polymer dictates the effective combination of water and ethylene glycol required, as shown in the comparison of methylcellulose and hydroxypropylmethylcellulose.

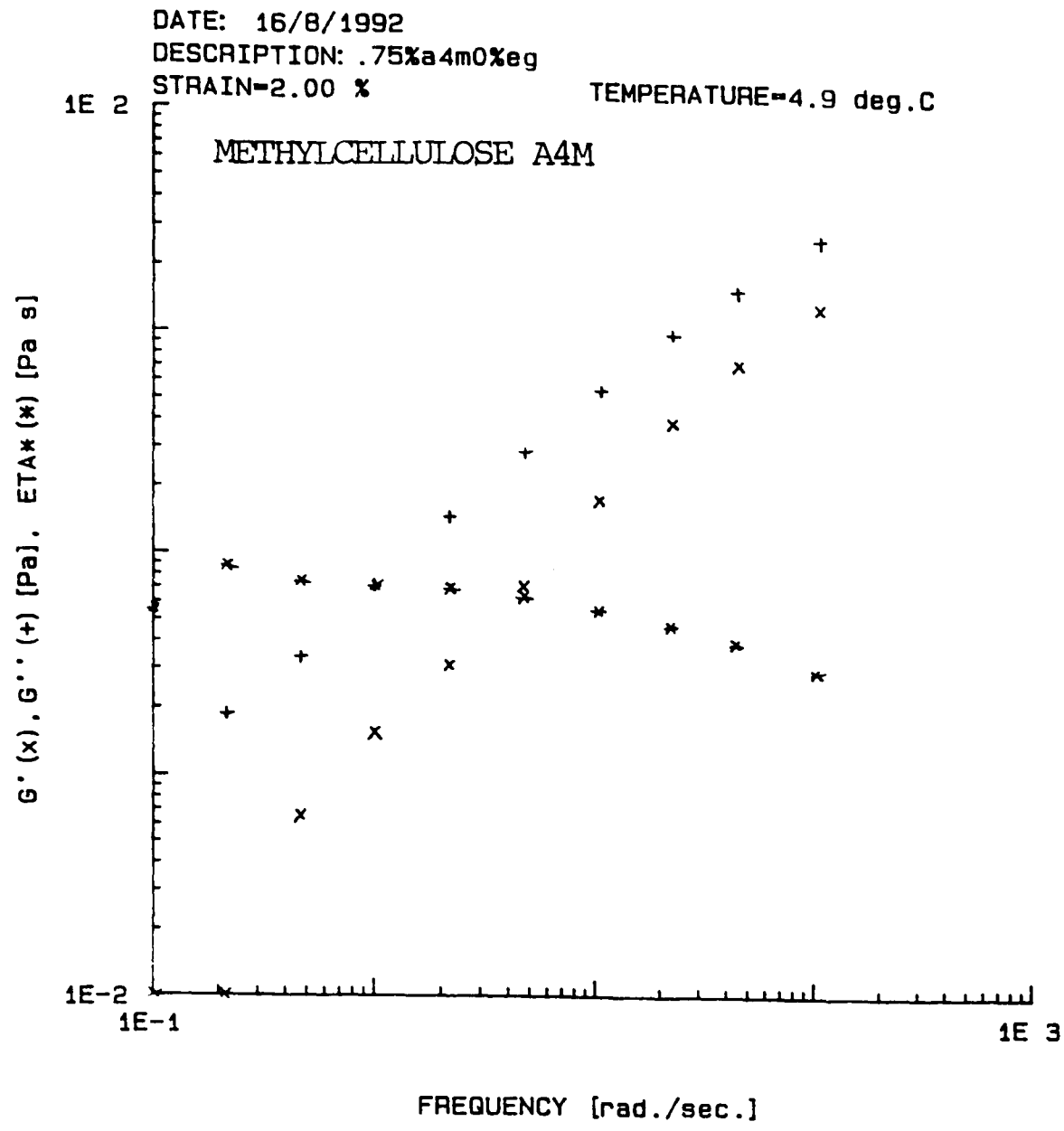
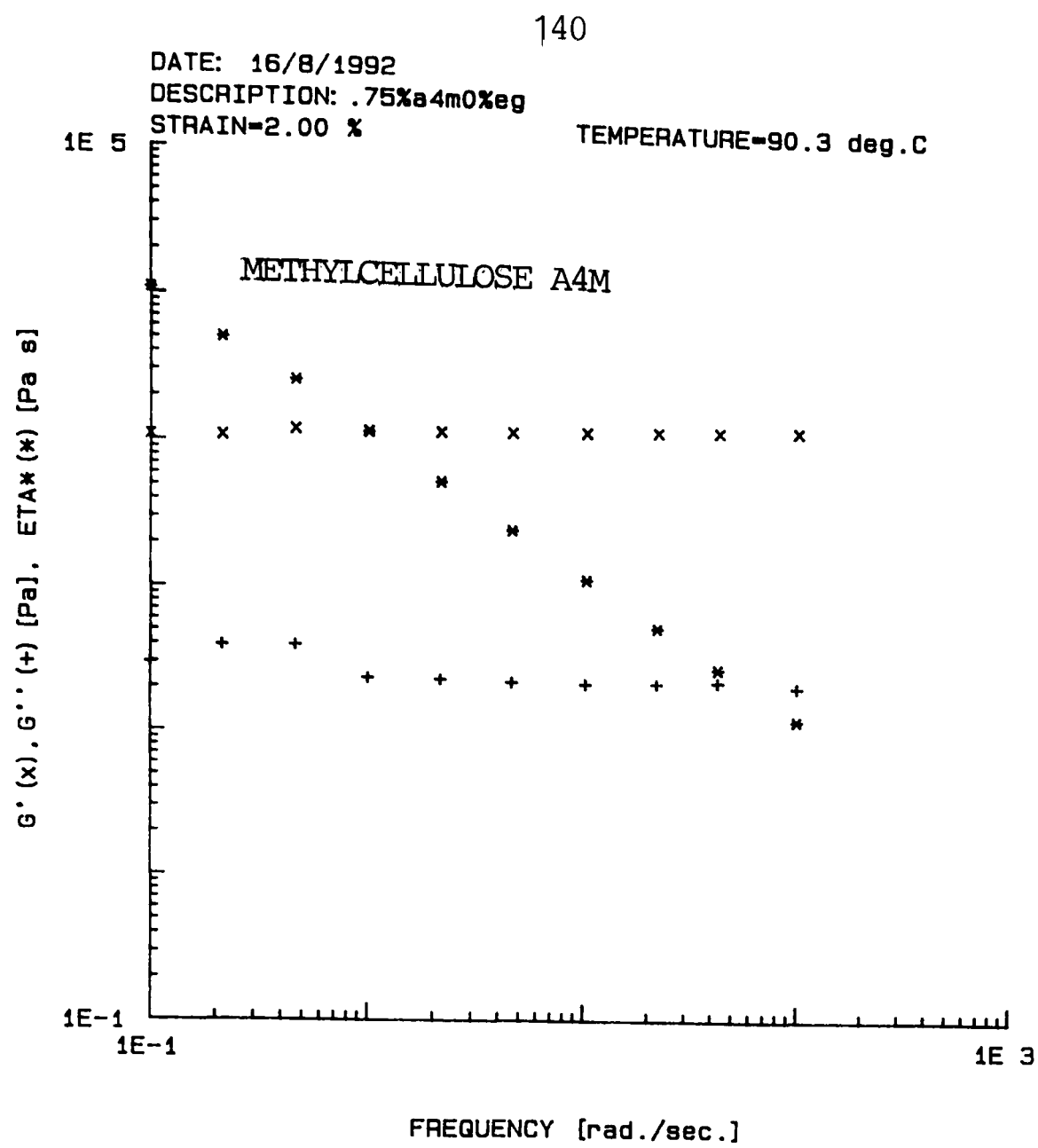


Figure 5.27 Comparison of the frequency dependence of G', G" and η^*, for methylcellulose (A4M) and hydroxypropylmethylcellulose (K4M), at 5°C in water.



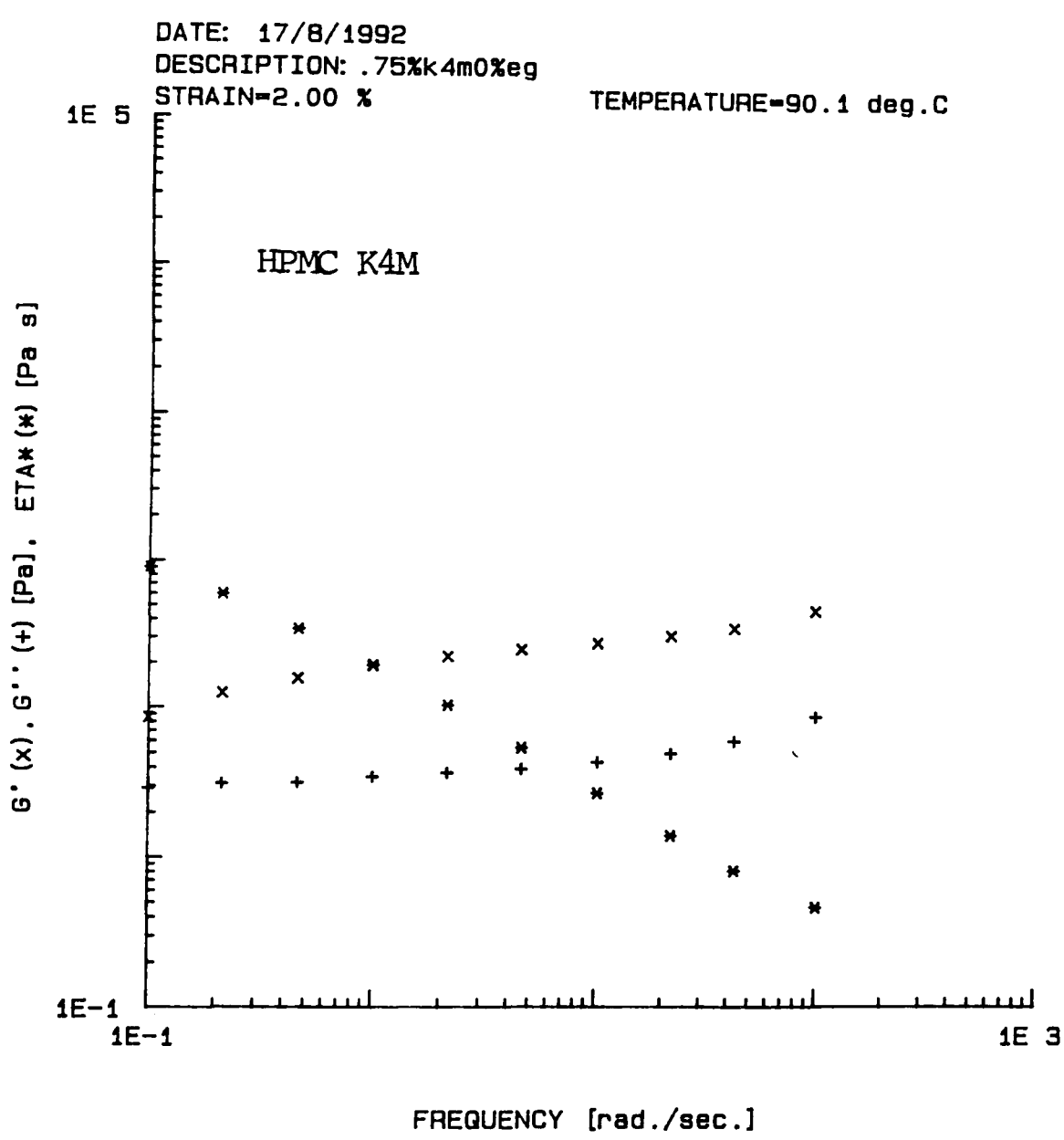
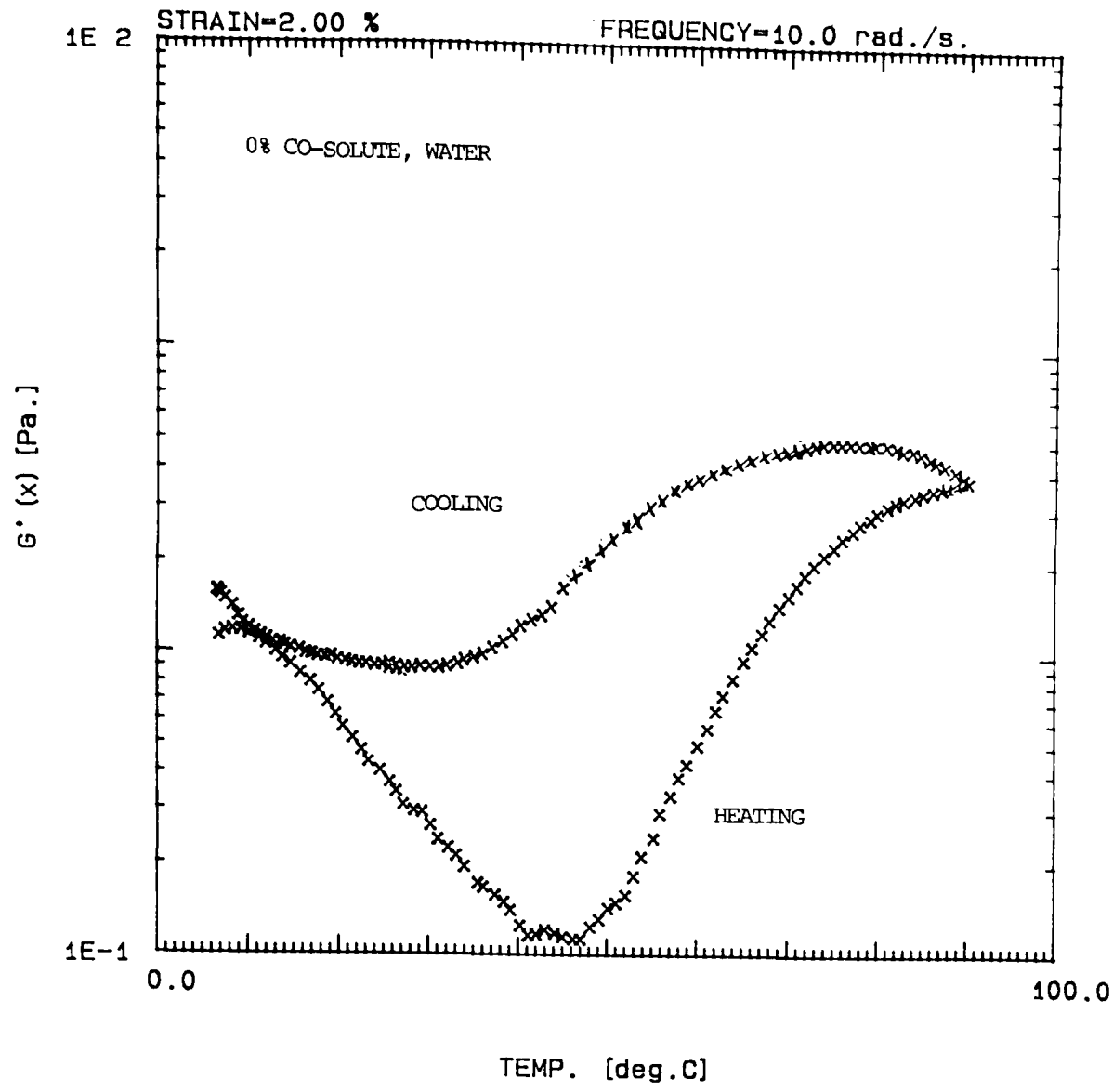


Figure 5.28 Comparison of the frequency dependence of G', G" and η^*, for methylcellulose (A4M) and hydroxypropylmethylcellulose (K4M), at 90°C in water.



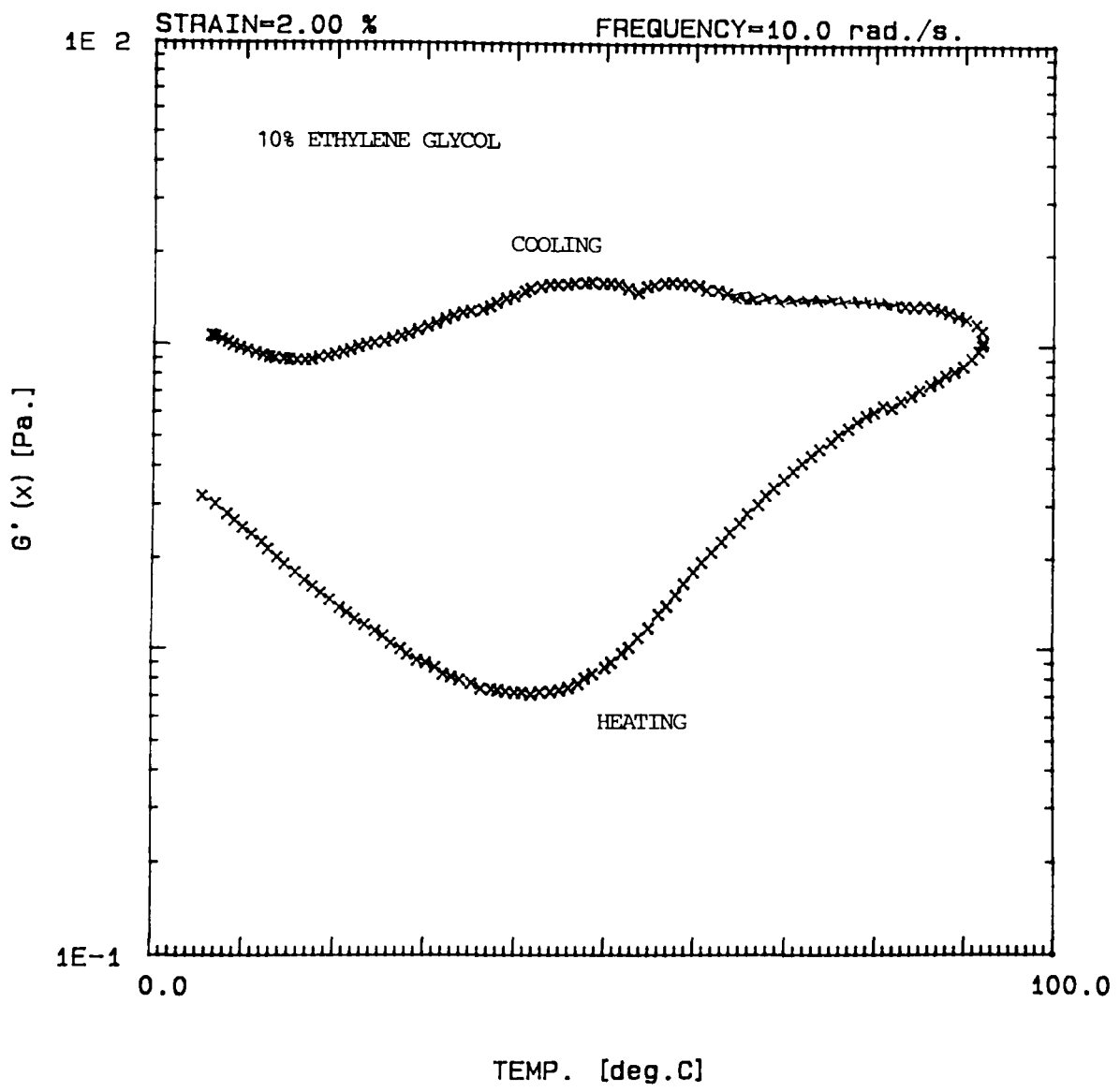


Figure 5.29 Comparison of the mechanical spectra of HPMC (K4M) in water and in 10% w/w ethylene glycol on heating and cooling.

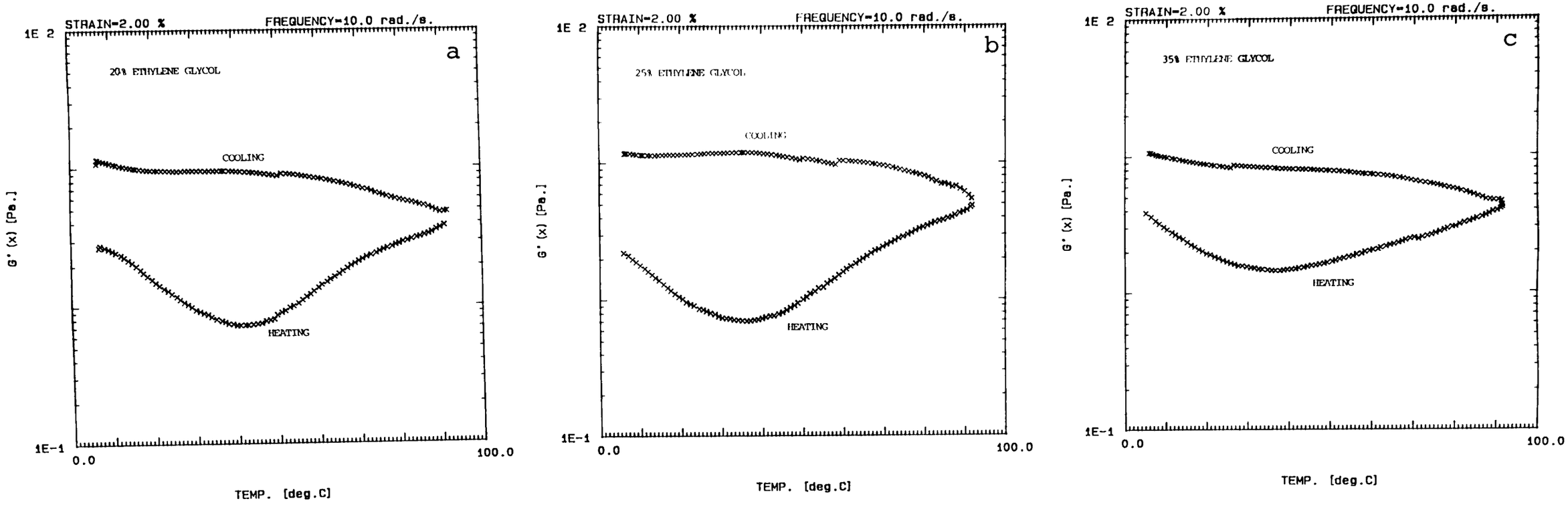


Figure 5.30 Comparison of the mechanical spectra of HPMC (K4M) with a) 20, b) 25 and c) 35% w/w ethylene glycol on heating and cooling.

REFERENCES

Arnott,S., Fumera,A., Scott,W.E., Dea,I.C.M., Moorhouse,R., Rees,D.A. (1974) *J. Mol. Biol.*, **90**, 269-284.

Aspinall,G.O. (1970) In: *The Carbohydtrates, Chemistry, Biochemistry.* 2nd Ed,Vol IIb,(Eds. Pigman,W., Horton,D.), Chapter 39, 530-535.

Braconnet,M.H. (1825) Recherches sur un novel acide universellement repéndu dans tous les végétaux. *Ann.Chim.Phys.*, Ser.2, **28**, 173.

Borchard,W. (1983) In: *Chemistry and Technology of Water Soluble Polymers.* (Ed. Finch, C.A.), Plenum Press, New York and London, Capter 6, 113-124.

Bryce,T.A., McKinnon,A.A., Morris,E.R., Rees,D.A., Thom,D. (1974) *Faraday Discuss. Chem. Soc.*, **57**, 221-229.

Buhl,S. (1990) In: *Gumms and Stabilsers for the Food Industry, 5.* (Eds. Phillips,G.O., Willims,P.A.), IRL Press at Oxford University Press, Oxford, NewYork, Tokyo, Chapter 4, 209-221.

Burchard,W. (1983) In: *Chemistry and Technology of Water Soluble Polymers.* (Ed. Finch, C.A.), Plenum Press, New York and London, Capter 7, 125-142.

Christensen,S.H. (1986) In: *Food Hydrocolloids, Vol III.* (Eds. Glicksman,M.), CRC Press Inc., Florida, Chapter 9, 205-230.

Coultate,T.P. (1989) In: *Food the Chemistry of it's Components, 2nd.Ed.* Royal Society Of Chemistry, Chapter 3.

Danishevsky,I., Whistler,F.A., Bettelheim,F.A. (1970) In: *The Carbohydrates, Chemistry, Biochemistry,* 2nd Ed, Vol IIA. (Eds. Pigman,W., Horton,D.).

Davis,M.A.F., Gidley,M.J., Morris,E.R., Powell,D.A., Rees,D.A. (1980) *Int. J. Biol. Macromol.*, Vol 2, October.

Dea,I.C.M., McKinnon,A.A., Rees,D.A. (1972) *J. Mol. Biol.*, **68**, 153-172.

Dea,I.C.M., Morris,E.R., Rees,D.A., Welsh,E.J., Barnes,H.A., Price,J. (1977) *Carbohydr. Res.*, **57**, 249-272.

Desmaris,A.J. (1973) In: *Industrial Gums.*(Ed. Whistler,R.L.), 2nd Ed, Academic Press, New York, Chapter XXIX, 649-672.

Durand,D., Bertrand,C., Clarke,A.H., Lips,A. (1990) *Int. J. Biol. Macromol.,* Vol 12, February.

Francis,B.J., Bell,J-M.K. (1975) *Trop. Sci.*, **17**, 1, 25-44.

Franks,F. (1975) In: *Water, a Comprehensive Treatise.* (Ed. Franks,F.) Plenum Press, New York and London, Vol., 4, Chapter 1.

Franks,F. (1983) In: *Chemistry and Technology of Water Soluble Polymers.* (Ed. Finch, C.A.), Plenum Press, New York and London, 157-178.

Franks,F. (1983) In: *Water.* The Royal Society of Chemistry, London, Chapter 9, 35-56.

Gelman,R.A. (1981) In: *Chemistry, Biochemistry and Application.* (Eds. Kennedy,J.F., Phillips,G.O., Wedlock,D.J., Williams,P.A.), Ellis Horward, Chapter 25, 293-300.

Gidley,M.J., Morris,E.R., Murray,E.J., Powell,D.A., Rees,D.A. (1980) *Int. J. Biol. Macromol.,* Vol 2, October.

Glicksman,M. (1969) In: *Food Science and Technology. A Series of Monographs.*(Ed. Glicksman,M.), Academic Press, New York and London, Chapter 12, 398-471.

Glicksman,M. (1982) In: *Food Hydrocolloids. Vol I.* (Ed. Glicksman,M.), CRC Press Inc., Florida, Chapter 3, Part IV, 64-72.

Glicksman,M. (1986) In: *Food Hydrocolloids. Vol III.* (Ed. Glicksman,M.), CRC Press Inc., Florida, 3-8.

Gordon,I.R., Krishan Akumar,V. (1990) IFINR., **3**, 30-33.

Grant,G.T., Morris,E.R., Rees,D.A., Smith,P.J.C., Thom,D. (1973) FEBS Letters, **32**, 195-198.

Greminger,G.K., Savage,A.B. (1973) In: *Industrial Gums*. (Ed. WHistler,R.L.), Academic Press, New York, Chapter XXVIII, 619-647.

Grover,J.A. (1986) In: *Food Hydrocolloids. Vol III*. (Ed. Glicksman,M.), CRC Press Inc., Florida, Chapter 4, 121-159.

Haque,A., Morris,E.R., Richardson,R.K. (1993) In: *Frontiers in Carbohydrate Reasearch -3*. In Press.

Hemminger,W., Höhne,G. (1984) In: *Calorimetry. Fundamentals and Practice*. Verlag Chemie. GmbH. Weinheim, Chapter 6, 102-109.

Heyman,E. (1935) *Trans Faraday Soc.*, **31**, 846-864.

von Hippel, Schleich,T., (1969) In: *Biological Macromolecules*. (Eds. Timasheff,S.N., Fasman,G.D.), Marcel Dekker Inc., New York, Vol II.

van Holde,K.E. (1985) *Physical Biochemistry*. 2nd Ed. Prentice-Hall, N.J.

Hourdet,D., Muller,G. (1991) *Carbohydr. Polym.*, 409.

Johnson,W.C.Jr. (1987) *Adv. Carbohydr. Chem. Biochem.*, **45**, 73-124.

Kauzmann,W., Clogh,F.B., Tobias,I. (1961) *Tetrahedron.*, **13**, 57-105.

Kauzmann, W. (1959) In: *Advances in Protein Chemistry*. Vol. XIV. (Eds. Anfinsen,Jr.,C.B., Anson,M.L., Bailey,K., Edsall,J.T.), Academic Press, New York and London, Chapter 1, 1-63.

Klug,E.D. (1971) *J. Polym. Sci.*, Part C, Nov.36, 491-508.

Kohn,R. (1971) *Carbohydr. Res.*, **20**, 351-356.

Kohn,R. (1975) *Pure Appl. Chem.*, **42**, 371-397.

Kohn,R. (1987) *Carbohdr. Res.*, **160**, 343-353.

Kohn,R., Furda,I. (1967) *Collection Czechoslov. Chem. Commun.*, Vol **32**, 4470-4484.

Kravtchenko,T.P., Pilnik,W. (1990) In: *Gums and Stabilisers for the Food Industry, 5.* (Eds. Phillips,G.O., Wedlock,D.J., Williams,P.A.),IRL Press at Oxford University Press, Oxford, New York, Tokyo, 281-286.

Mackenzie,R.C., Layle,P.G. (1986) Thermal Analysis. In: *Chemistry in Britain.* November, 1005-1011.

Mitchell,J.R., Blanshard,J.M.V. (1976) *J. of Texture Studies.* **7**, 341-351.

Morawetz,H. (1975) Macromolecules in Solution. In: *High Polymers Interscience.* 2nd Ed., J. Wiley and Sons Inc., 21.

Morris,E.R. (1980) *Food Chemistry.* **6**, 15-39.

Morris,E.R. (1983) In: *Gums and Stabilisers for the Food Industry, 2.* (Eds. Phillips,G.O., Wedlock,D.J., Williams,P.A.), Elsevier, London, Chapter 2, 57-78.

Morris,E.R. (1986) In: *Gums and Stabilisers for the Food Industry, 3.* (Eds. Phillips,G.O., Wedlock,D.J., Williams,P.A.), Elsevier, London, Chapter 1, 3-16.

Morris,E.R. (1986) *British Polymer Journal.* Vol **18**, No. 1.

Morris,E.R. (1987) In: *Industrial Polysaccharides.* (Eds. Stivala,S.S., Crescenzi,V., Dea,I.C.M.), 431-457.

Morris,V.J. (1986) In: *Functional Properties of Food Macromolecules.* (Eds. Mitchell,J.R., Ledward,D.A.), Elsevier Applied Science Publishers, London and New York, Chapter 3, 121.

Morris,E.R., Cutler,A.N., Ross-Murphy,S.B., Rees,D.A. (1981) *Carbohydr. Polym.*, **1**, 5-21.

Morris,E.R., Frangou,S. (1981) *Techniques in Carbohydrate Metabolism.*, **B308**, 1-51.

Morris,E.R., Gidley,M.J., Murray,E.J., Powell,D.A. (1980) *Int. J. Biol. Macromol.*, Vol 2, October, 327-334.

Morris,E.R., Norton,I.T. (1983) In: *Aggregation Processes in Solution.* (Eds. Wyn-Jones,E., Gormally,J.), Elsevier, Amsterdam, 549-593.

Morris,E.R., Powell,D.A., Gidley,M.J., Rees,D.A. (1982) *J. Mol. Biol.*, **155**, 507-516.

Morris,E.R., Rees,D.A., Robinson,G. (1980) *J. Mol. Biol.*, **138**, 349-362.

Morris,E.R., Rees,D.A., Robinson,G., Young,G.A. (1980) *J. Mol. Biol.*, **138**, 363-374.

Morris,E.R., Rees,D.A., Thom.D., Boyd,J. (1978) *Carbohydr. Res.*, **66**, 145-154.

Morris,E.R., Ross-Murphy,S.B. (1981) *Techniques in Carbohydrate Metabolism.* **B139**, 1-46.

Owens,H.S., Lotzkar,H., Schultz,T.H., Maclay,W.D. (1946) *J. Am. Chem. Soc.*, **68**, 1628.

Padival,R.A., Ranganna,S., Manjrekar,S.P. (1979) *J. Fd. Technol.*, **14** , 277-287.

Pilnik,W. (1990) In: *Gums and Stabilisers for the Food Industry,5.* (Eds. Phillips,G.O., Wedlock,D.J., Williams,P.A.), IRL Press at Oxford University Press, Oxford, New York, Tokyo.

Plaschina,I.G., Braudo,E.E., Tolstoguzov,V.B. (1978) *Carbohdyr. Res.*, **60**, 1-8.

Plaschina,I.G., Muratalieva,I.R., Braudo,E.E., Tolstoguzov,V.B. (1986) *Carbohdr. Polym.*, **6**, 15-34.

Powell,D.A., Morris,E.R., Gidley,M.J., Rees,D.A. (1982) *J. Mol. Biol.* **155**, 517-531.

Rees,D.A. (1969) *Adv. Carbohdr. Chem. Biochem.*, Vol **24**, 267-332.

Rees,D.A. (1972) *Chemistry and Industry*. 630-636.

Rees,D.A. (1977) In: *Polysaccharide Shapes*. Chapman and Hall Ltd., London.

Rees,D.A. (1972) *Biochem. J.*, **126**, 257-273.

Rees,D.A. (1982) *Carbohdr. Polym.*, **2**, 254-263.

Rees,D.A., Morris,E.R., Thom,D., Madden,J.K. (1982) In: *The Polysaccharides, Vol I.* (Ed. Aspinall,G.O.), Academic Press, New York, Chapter 5, 195-290.

Rees,D.A., Wight,A.W. (1971) *J. Chem. Soc.*, **B**, 1365-1372.

Richardson,R.K. (1991) PhD thesis, Department of Food Research and Technology, Cranfield Institute of Technology, Silsoe.

Robitalle,L., Turcote,N., Fortin,S., Charlet,G. (1991) *Macromolecules*. **24**, 2413-2418.

Rolin,C., DeVries,J. (1990) In: *Food Gels*. (Ed. Harris,P.), Elsevier Applied Science, London and New York, Chapter 10, 401-434.

Ross-Murphy,S.B. (1988) In: *Food Structure It's Creation and Evaluation*.(Eds. Blanshard,J.M.V., Mitchell,J.R.), Butterworth, G.B., Chapter 21, 387-400.

Sarkar,N. (1979) *J. Appl. Polym. Sci.*, **24**, 1073-1087.

Savage,A.B. (1957) *Ind. Eng. Chem.*, **49**, 99-103.

Seale,R. (1982) *Carbohdr. Res.*, **110**, 101-112.

Sinanoglu,O., Abdulnur,S. (1965) *Fed.Proc.*, **24**, S-12.

Smidsrod,O., Haug,A. (1971) *Biopolymers*, **10**, 1213-1227.

Stoddart,J.F. (1971) *Steroechemistry of Carbohydtrates*. J. Wiley and Sons Inc.

Stoddart,J.F., Spires,I.P.C., Tipton,K.F. (1969) *Biochem. J.*, **144** , 863.

Tanford,C. (1961) *Physical Chemistry of Macromolecules*. J. Wiley, New York.

Tanford,C. (1980) *The Hydrophobic Effect. Formation of Micelles and Biological Membranes*. 2nd Ed, J. Wiley and Sons Inc., New York.

Taylor,A.J., (1982) *Carbohydr. polym.*, **2**, 9-17.

Thibault,J-F., Rinaudo,M. (1985) *British Polym. J.*, Vol **17**, 2.

Thibault,J-F., Rinaudo,M. (1986) *Biopolymers*, Vol **25**, 455-468.

Thom,D., Dea,I.C.M., Morris,E.R., Powell,D.A. (1982) *Prog. Fd. Nutr. Sci.*, **6**, 97-108.

Toft,K. (1982) *Prog. Fd. Nutr. Sci.*, **6**, 89-96.

Towle,G.A., Christensen,O. (1973) In: *Industrial Gums*. (Ed. Whistler,R.L.), Academic Press, London and New York, 429-461.

Tuerena,C.E., Taylor,A.J., Mitchell,J.R. (1982) *Carbohdr. Polym.*, **2**, 193-203.

Walkinshaw,M.D., Arnott,S. (1981a) *J. Mol. Biol.*, **153**, 1055-1073.

Walkinshaw,M.D., Arnott,S. (1981b) *J, Mol. Biol.*, **153**, 1075-1085.

Walter,R.H., Talomie,T.G. (1990) *Food Hydrocolloids,* **4**, 197-203.

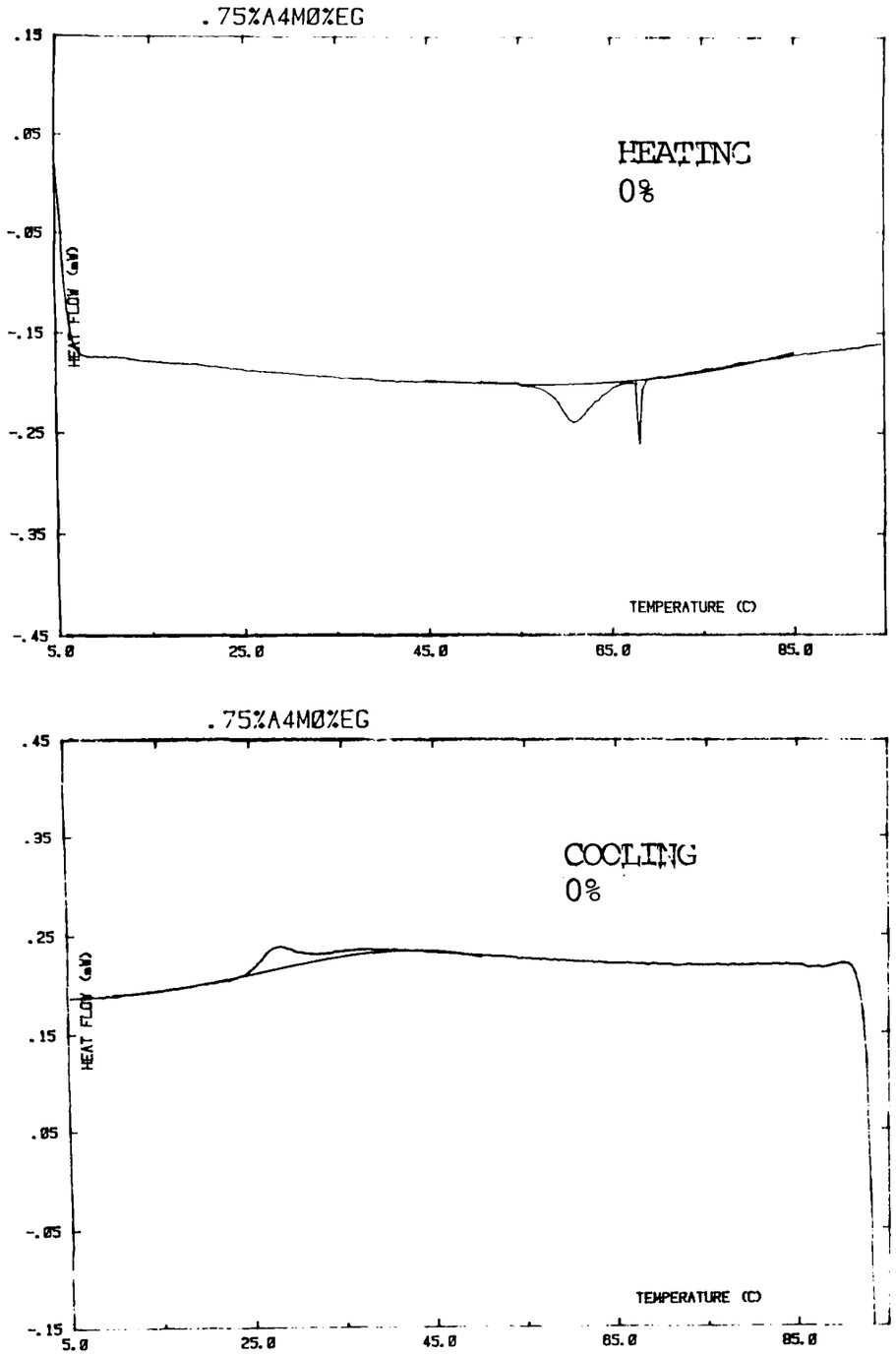
Werbowyj,R.S., Gray,G. (1980) *Macromolecules,* **13**, 69-73.

Wright,D.J. (1984) Thermoanalytical Methods in Food Research, In: *Biophysical Methods in Food Research, Vol 5*. (Ed.Chan,H.W-S.), Blackwell Scientific Publications, Oxford.

Wright,D.J. (1986) *Analytical Procedings*. November, **23**, 389-390.

Yamamoto,D., Naomichi,I. (1969) The sol-gel transformation of methylcellulose in water, In: *Meisi Daigaku Nagakubu Kenkyu Hokoku,* **24**, 37-42.

APPENDIX



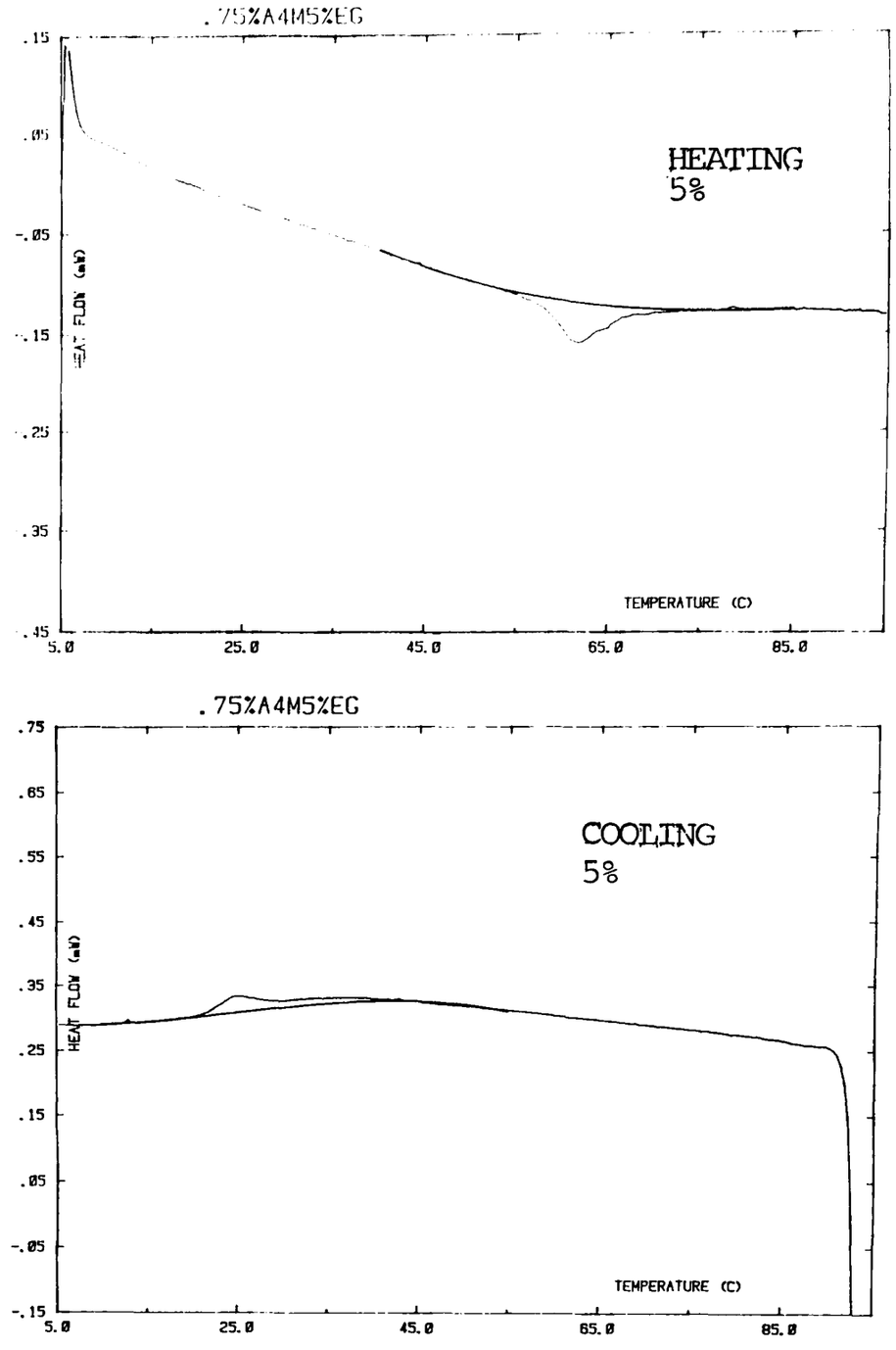
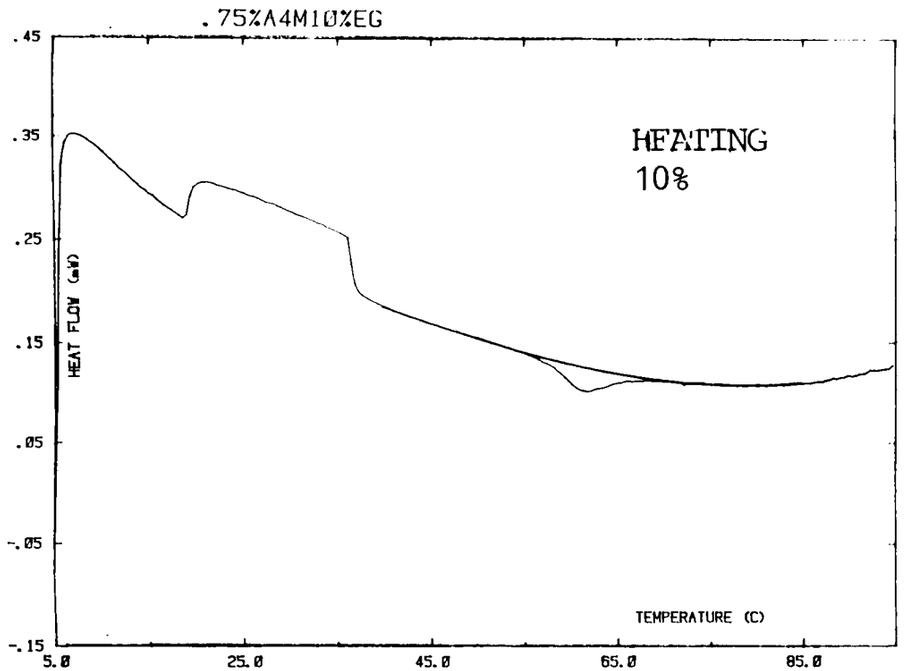
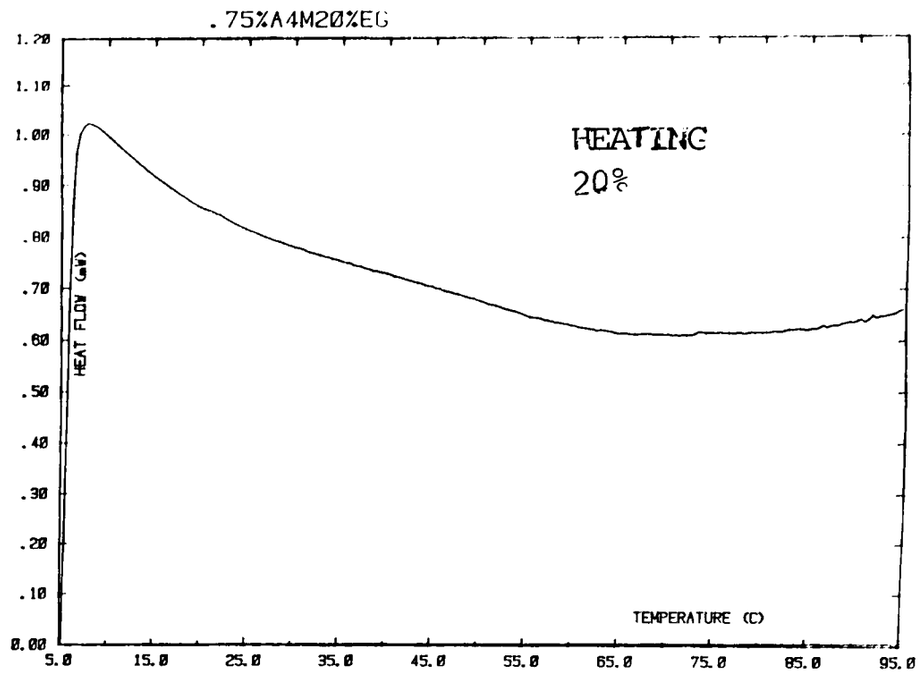
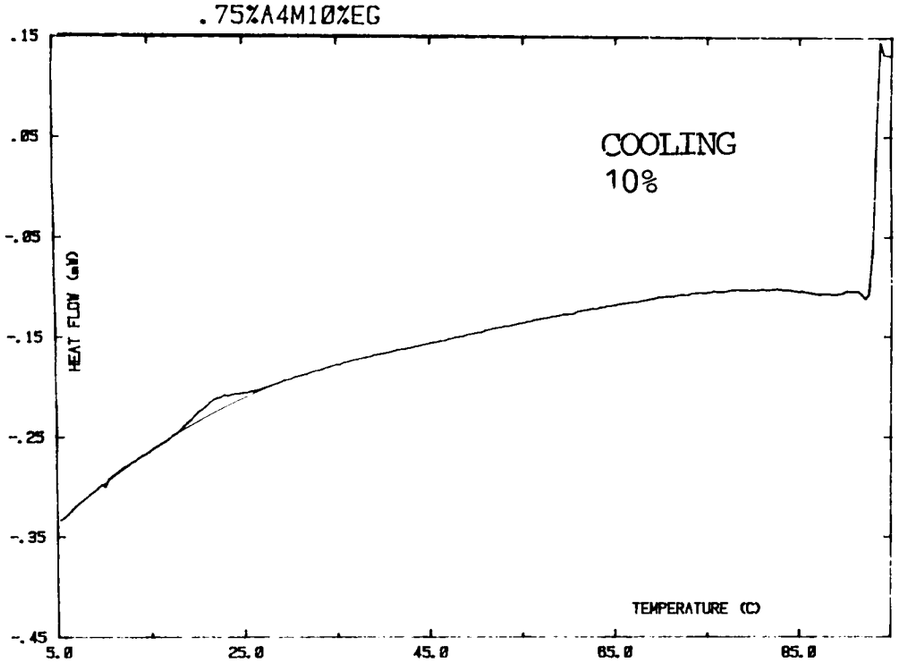


Figure 1 DSC scans of methylcellulose in 0% and 5% w/w ethylene glycol, scan rate 0.2°/min







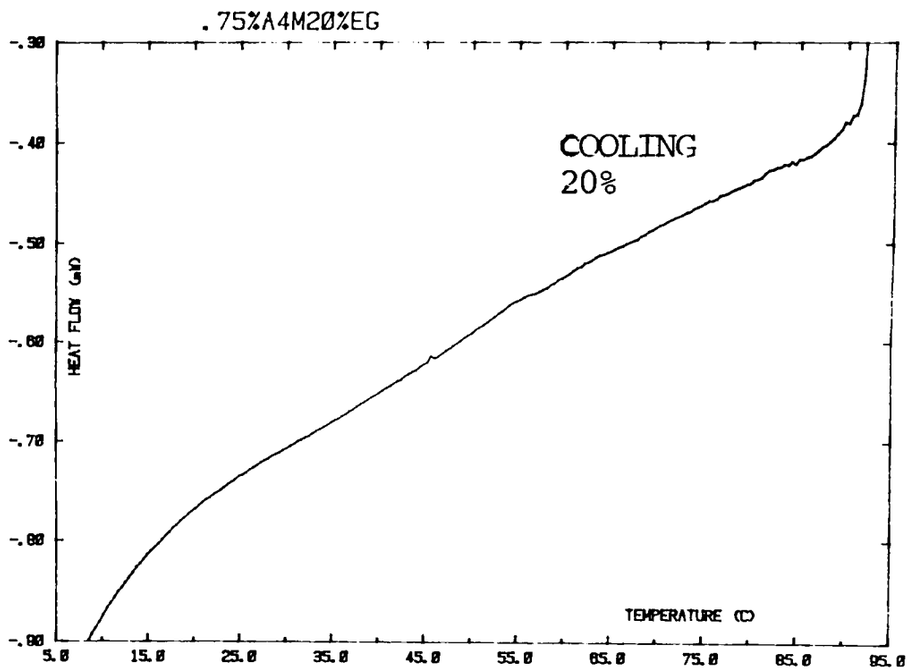
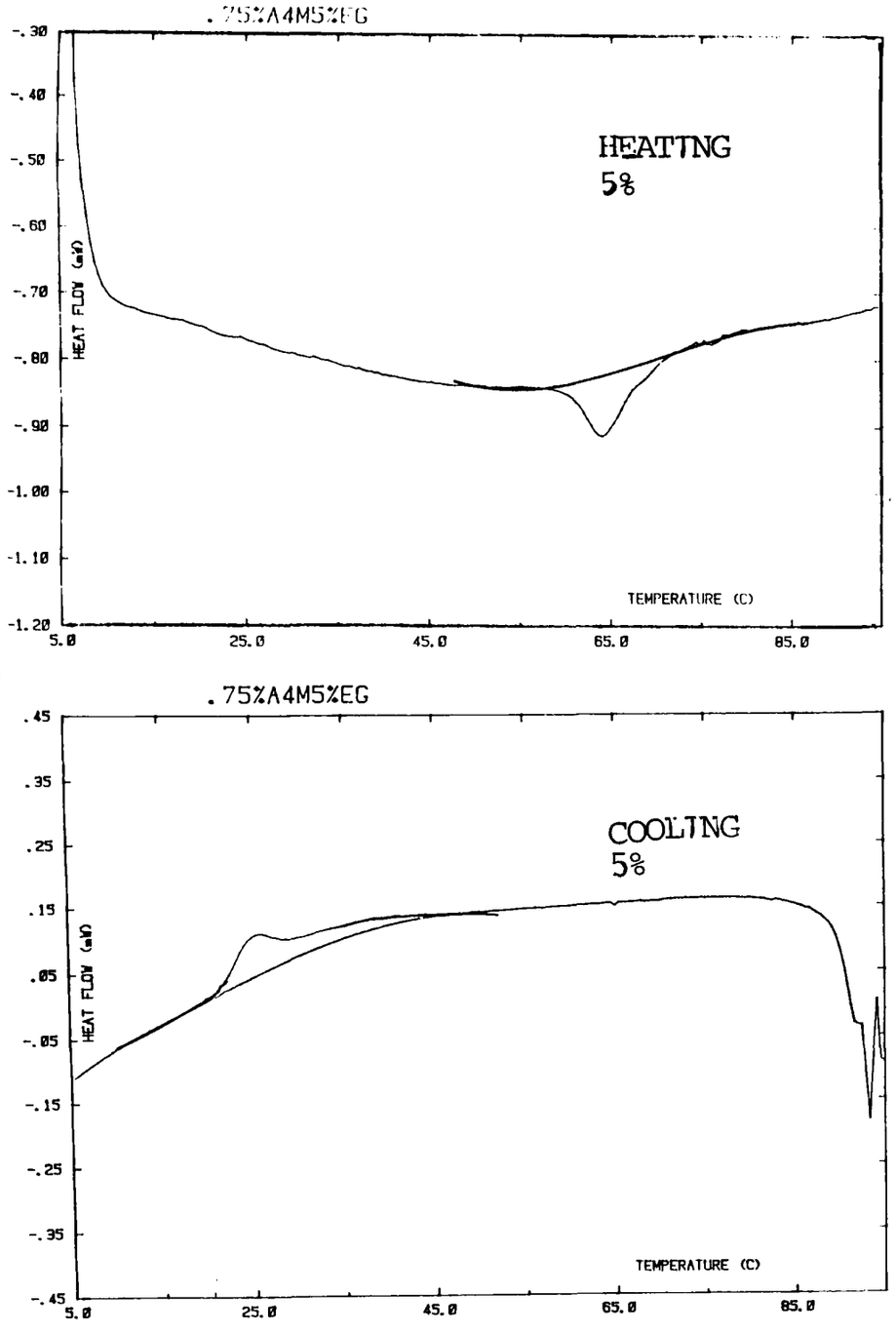


Figure 2 DSC scans of methylcellulose in 10% and 20% w/w ethylene glycol, scan rate 0.2°/min.



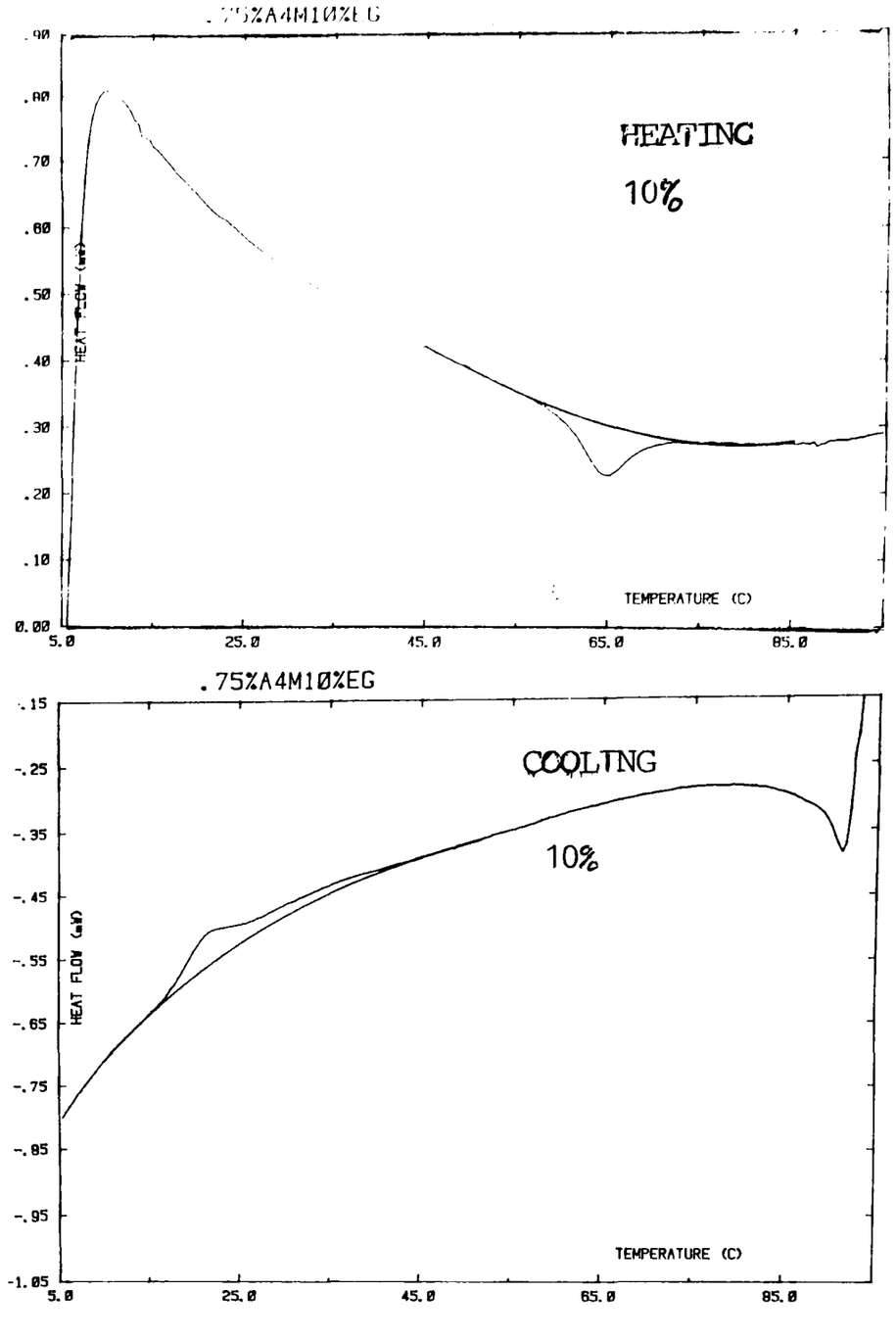
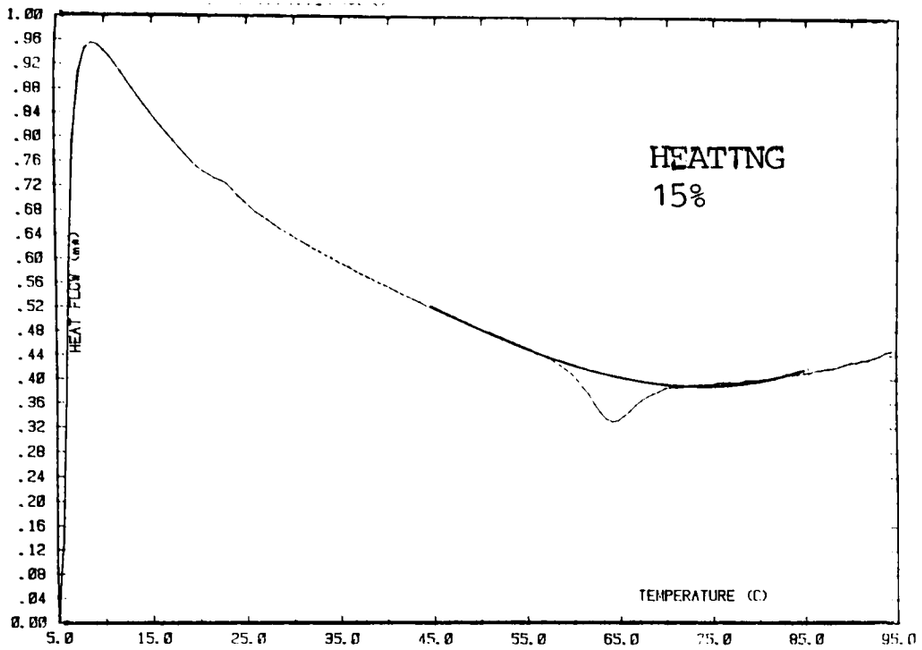
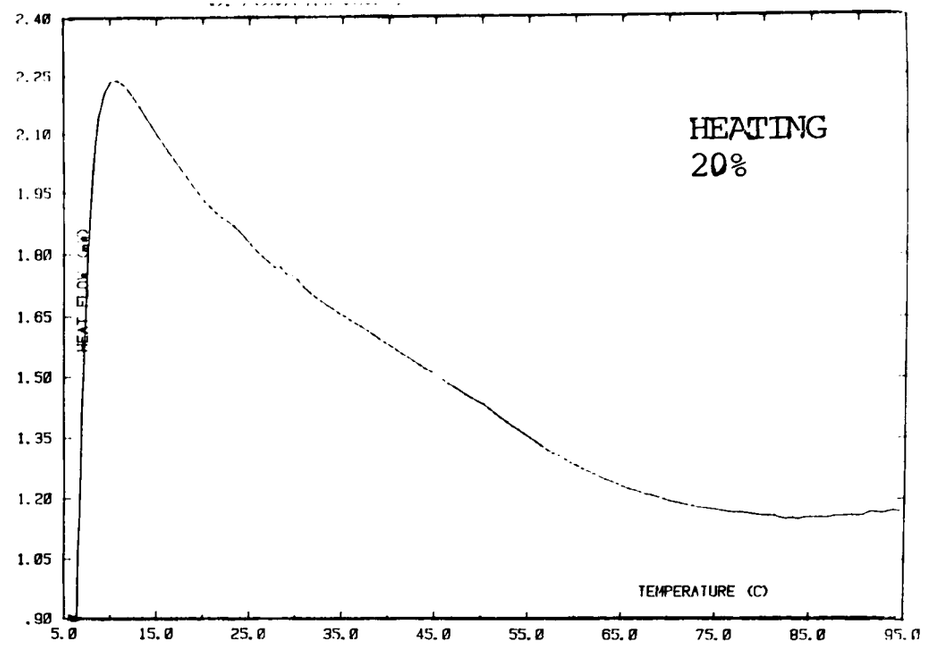
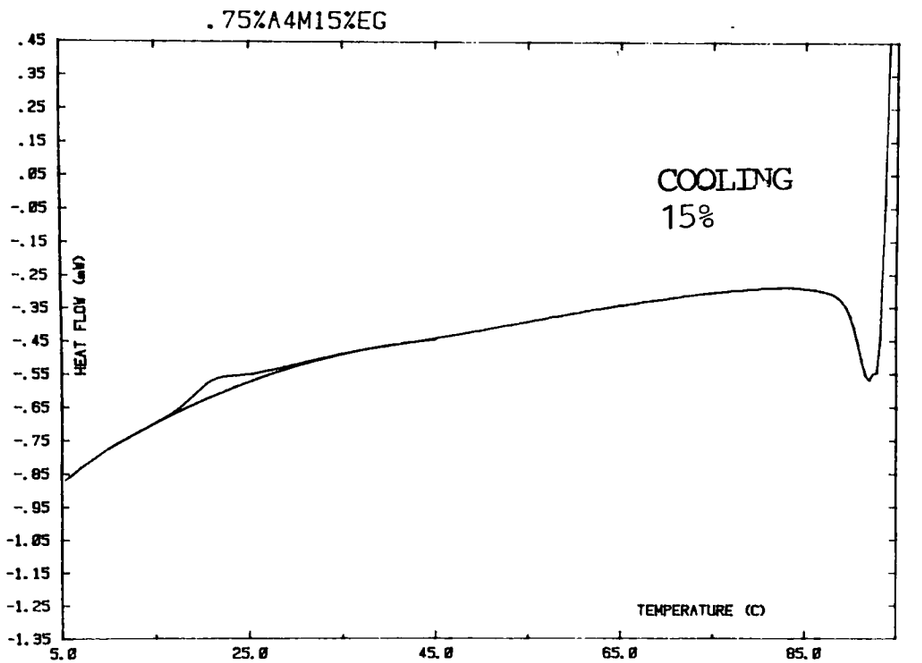


Figure 3 DSC scans of methylcellulose in 5% and 10% w/w ethylene glycol, scan rate







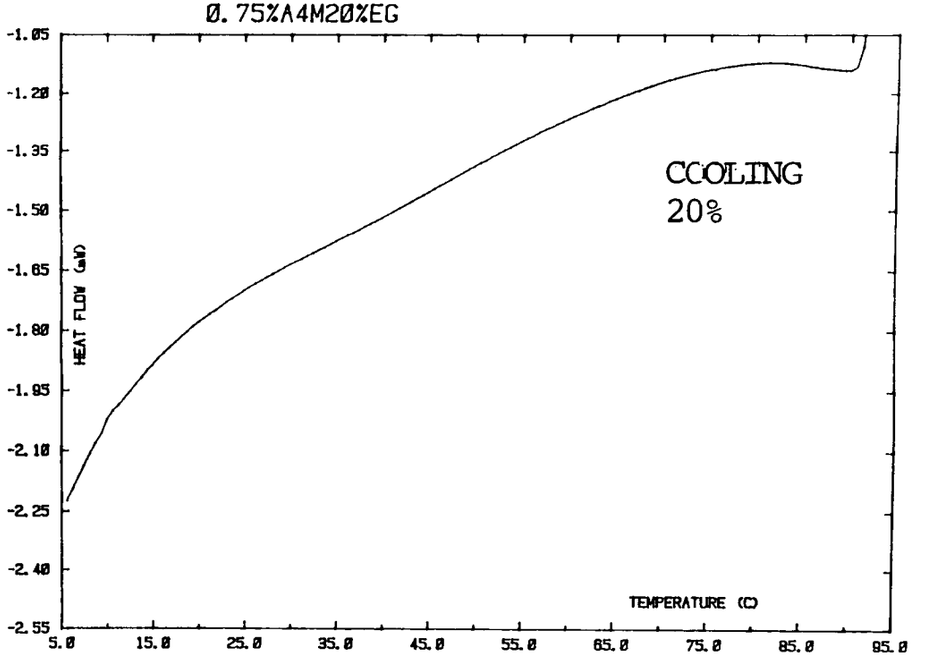
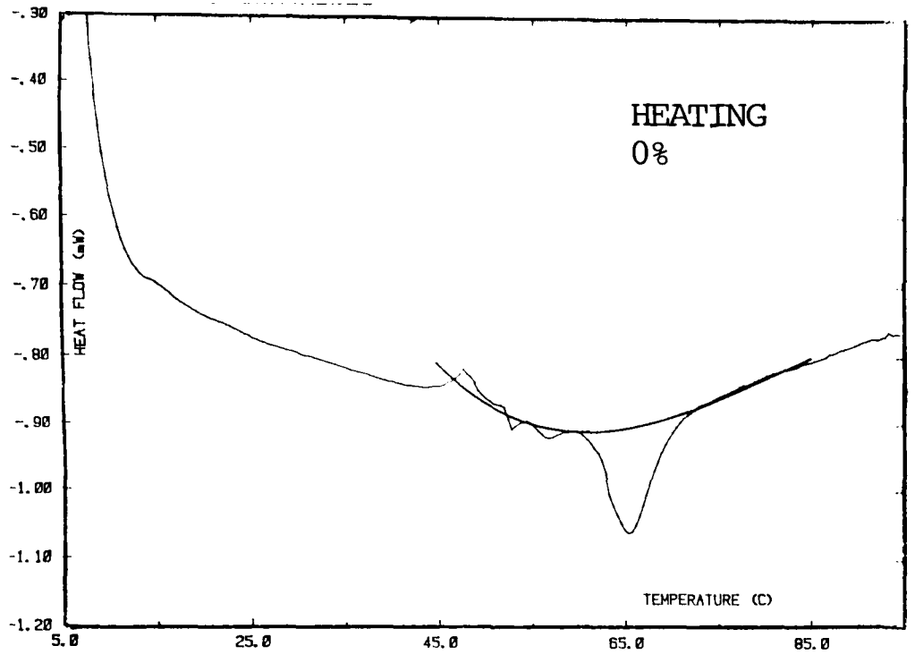
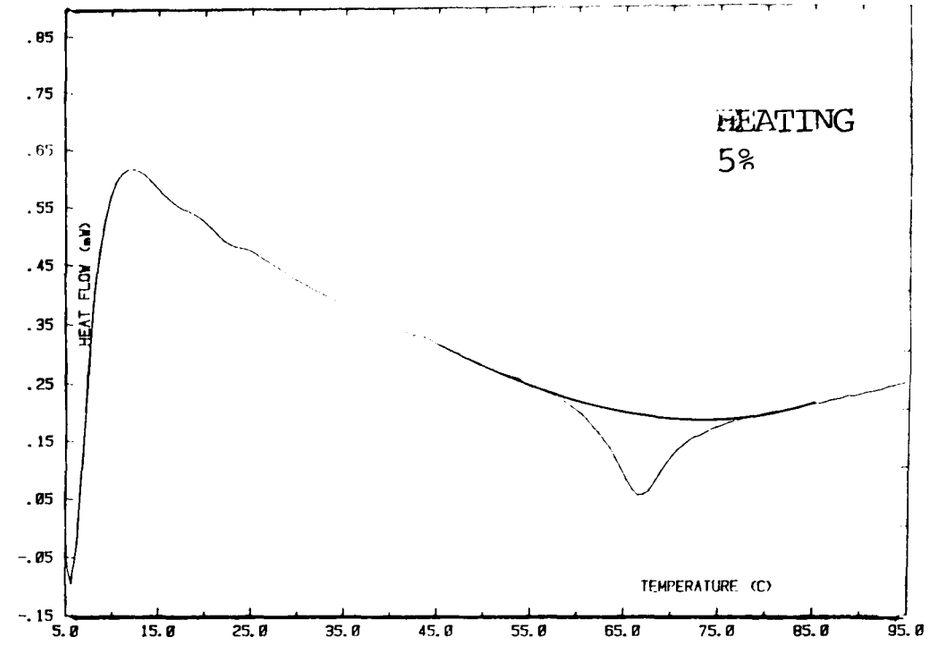


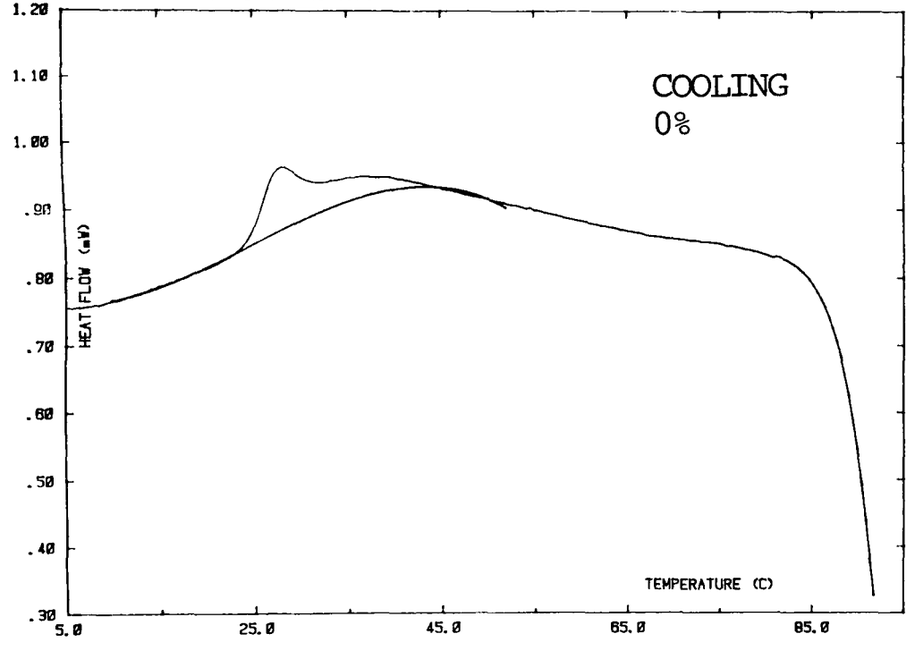
Figure 4 DSC scans of methylcellulose in 15% and 20% w/w ethylene glycol, scan rate 0.5°/min



.75%A4M0%EG



.75%A4M5%EG



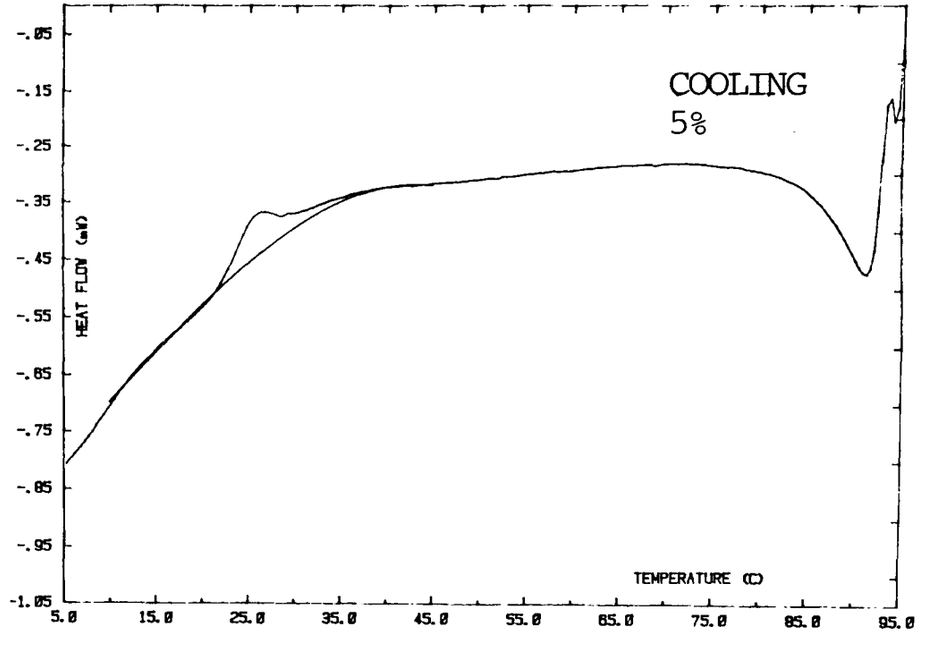
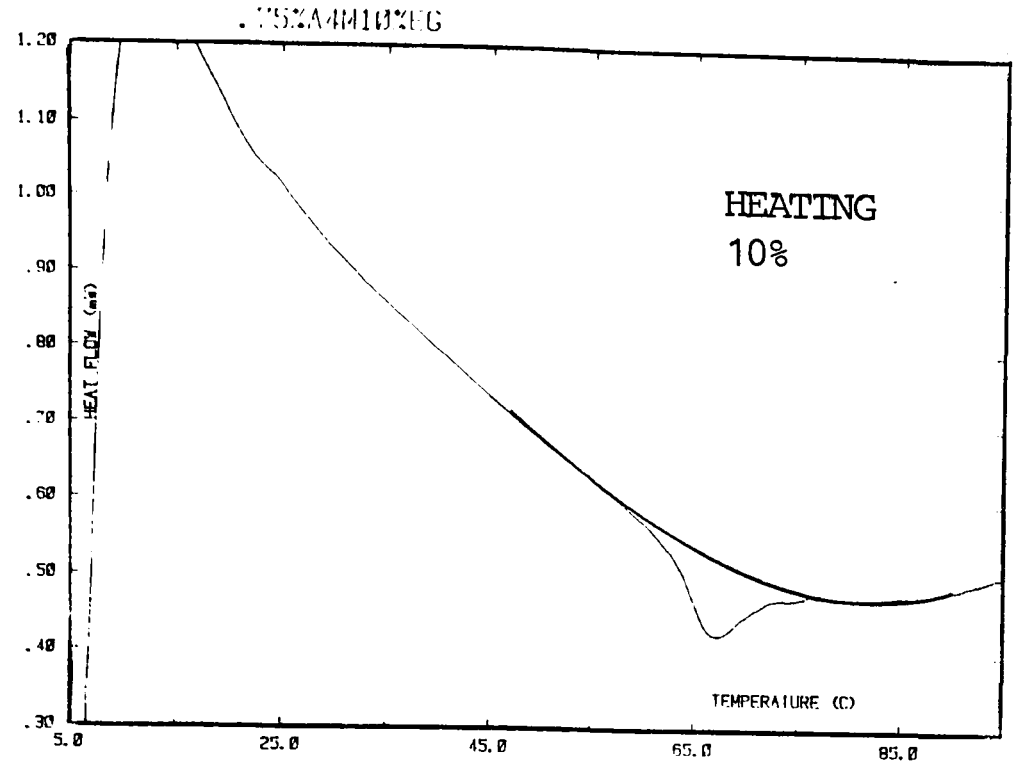


Figure 5 DSC scans of methylcellulose in 0% and 5% w/w ethylene glycol, scan rate



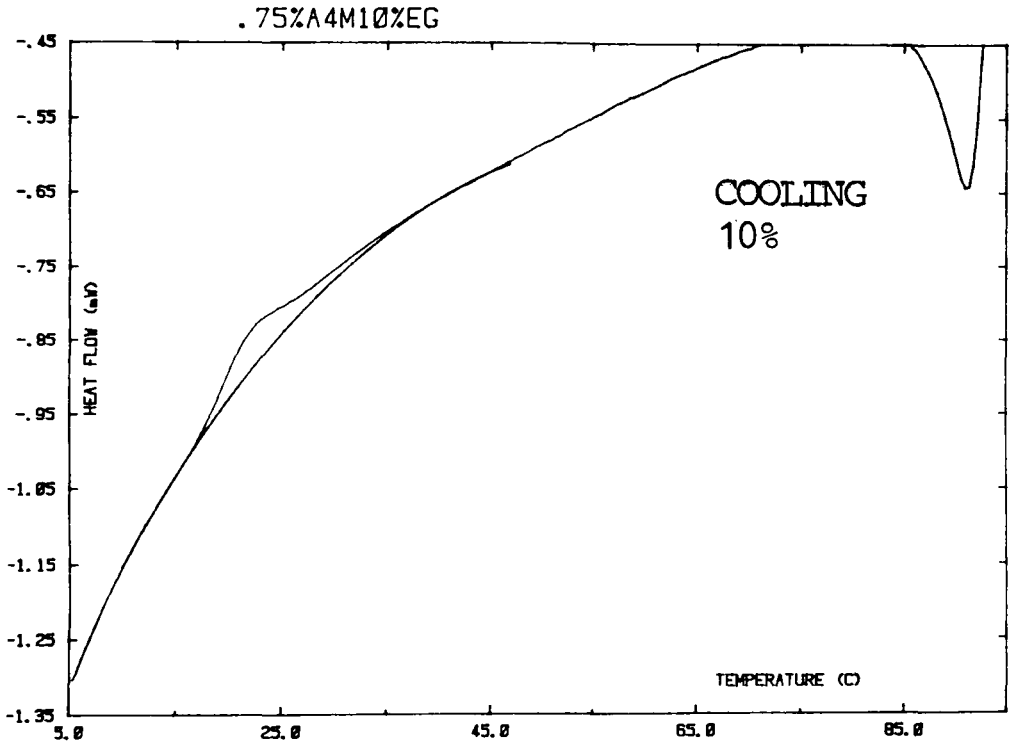
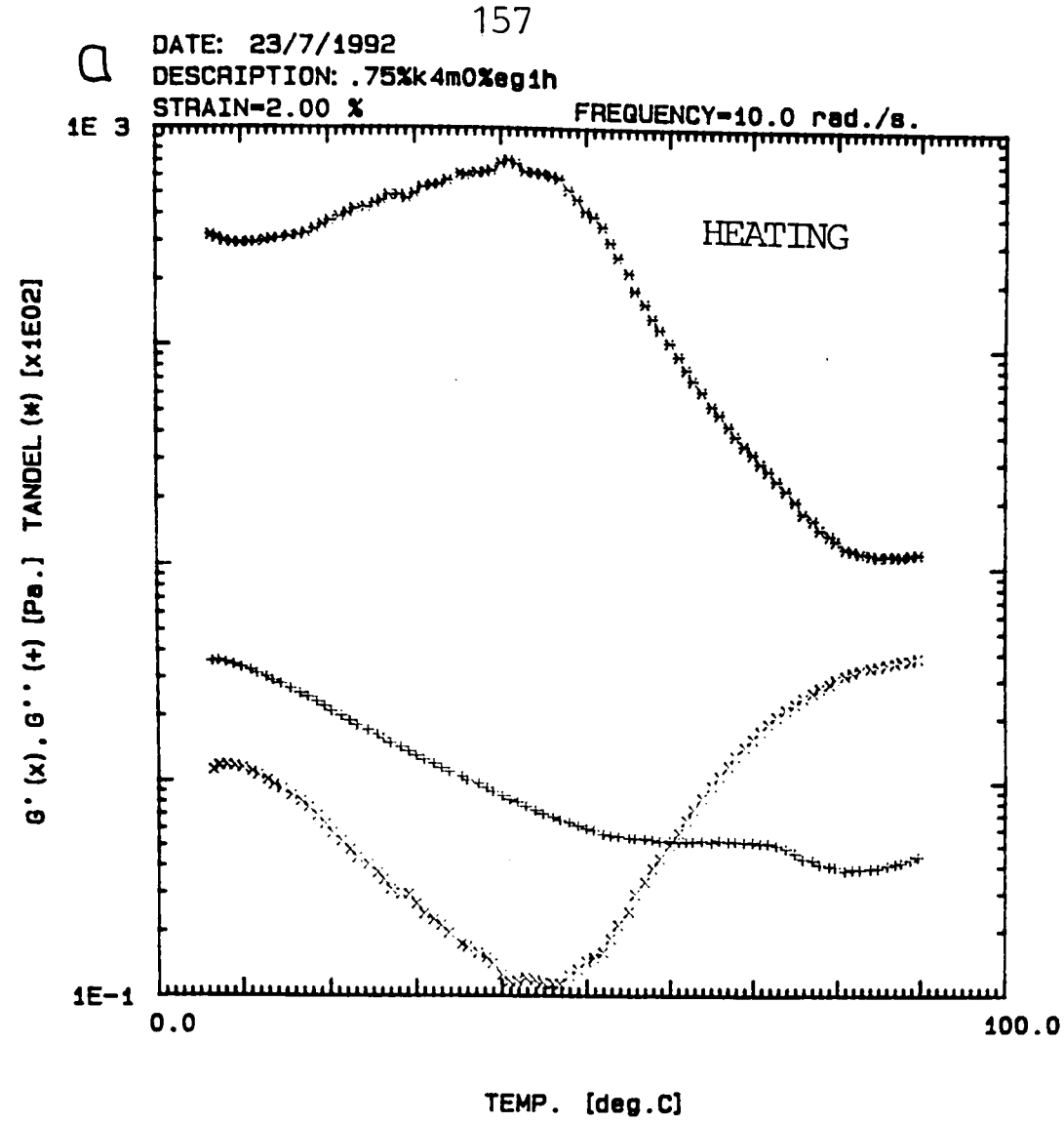


Figure 6 DSC scans of methylcellulose in 10% w/w ethylene glycol, scan rate 0.8°/min.



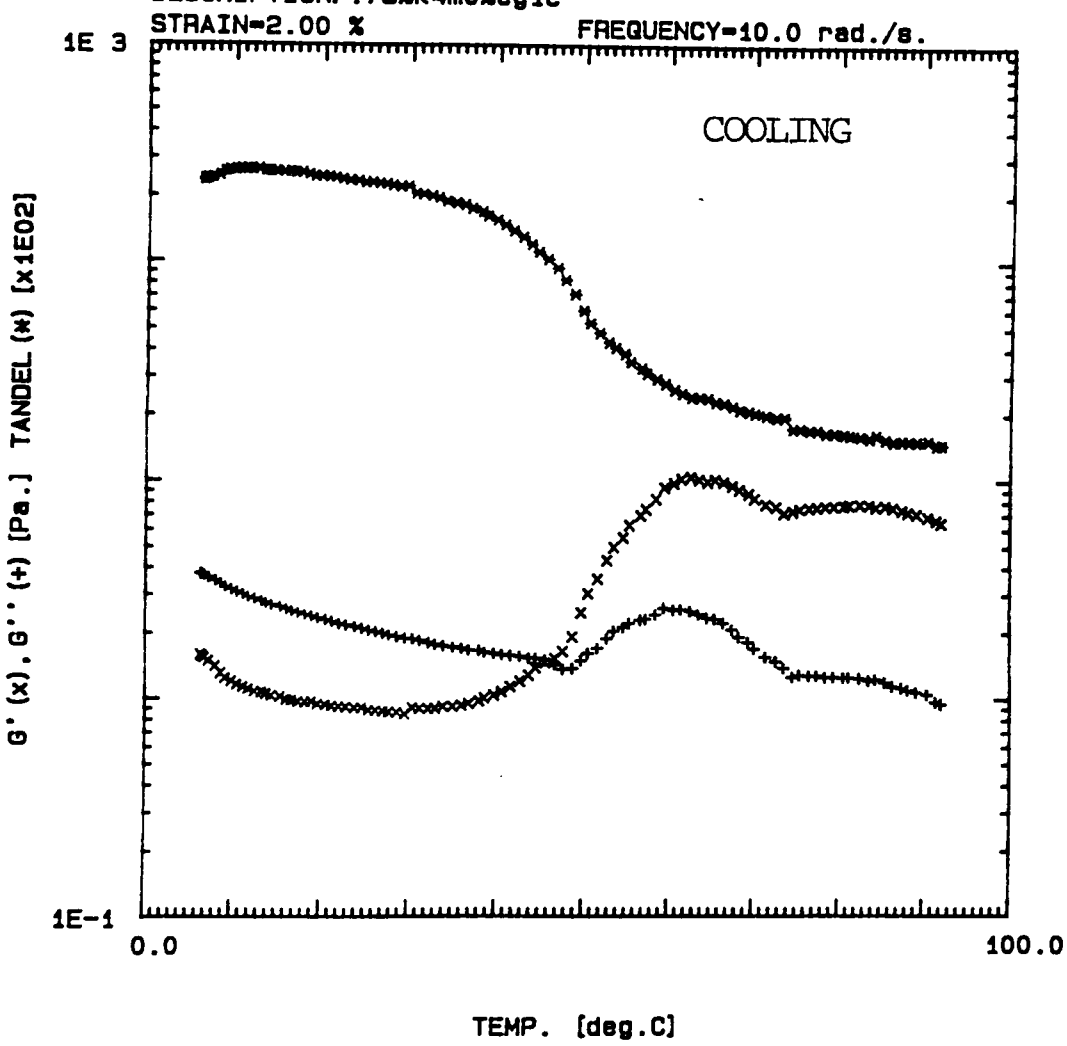
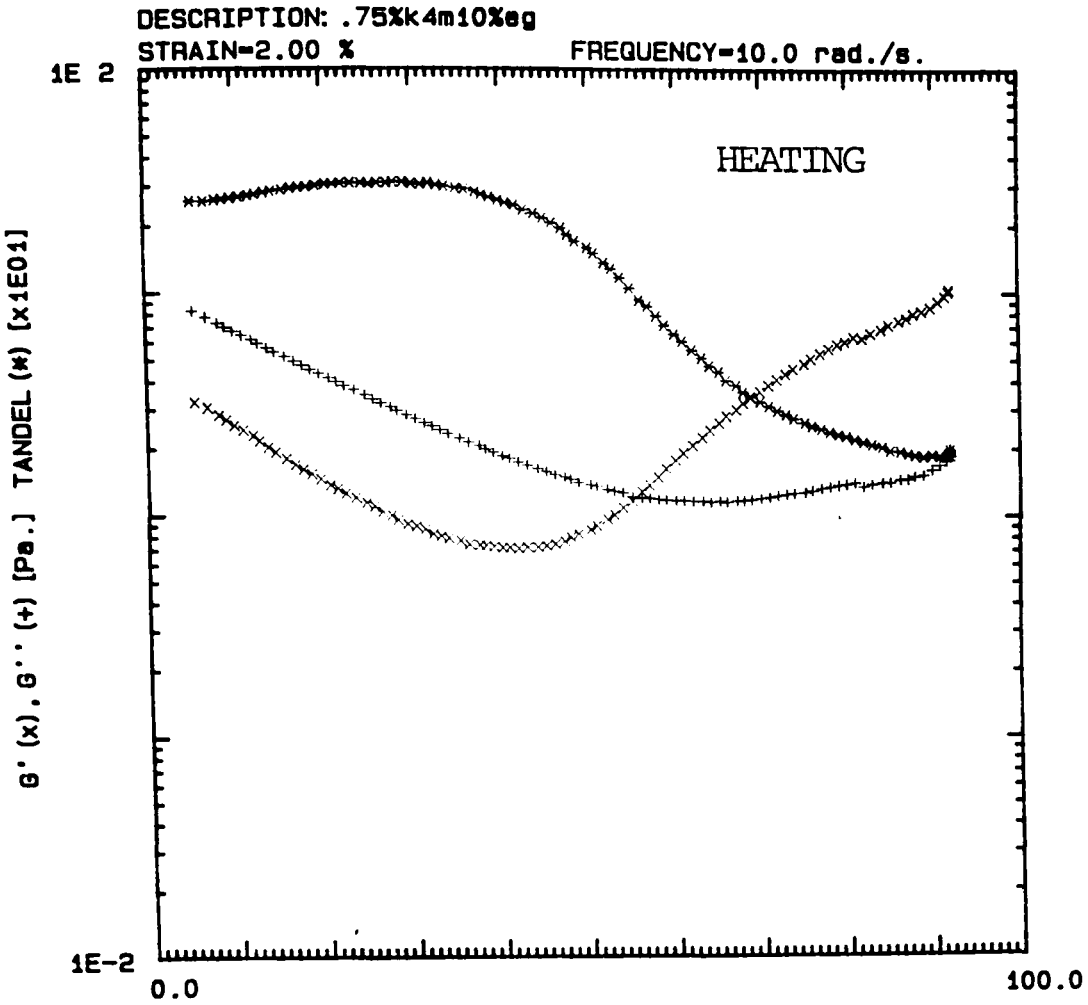


Figure 7 Changes in structural moduli of 0.75% hydroxypropylmethylcellulose, K4M with 0% ethylene glycol. a) heating, b) cooling.



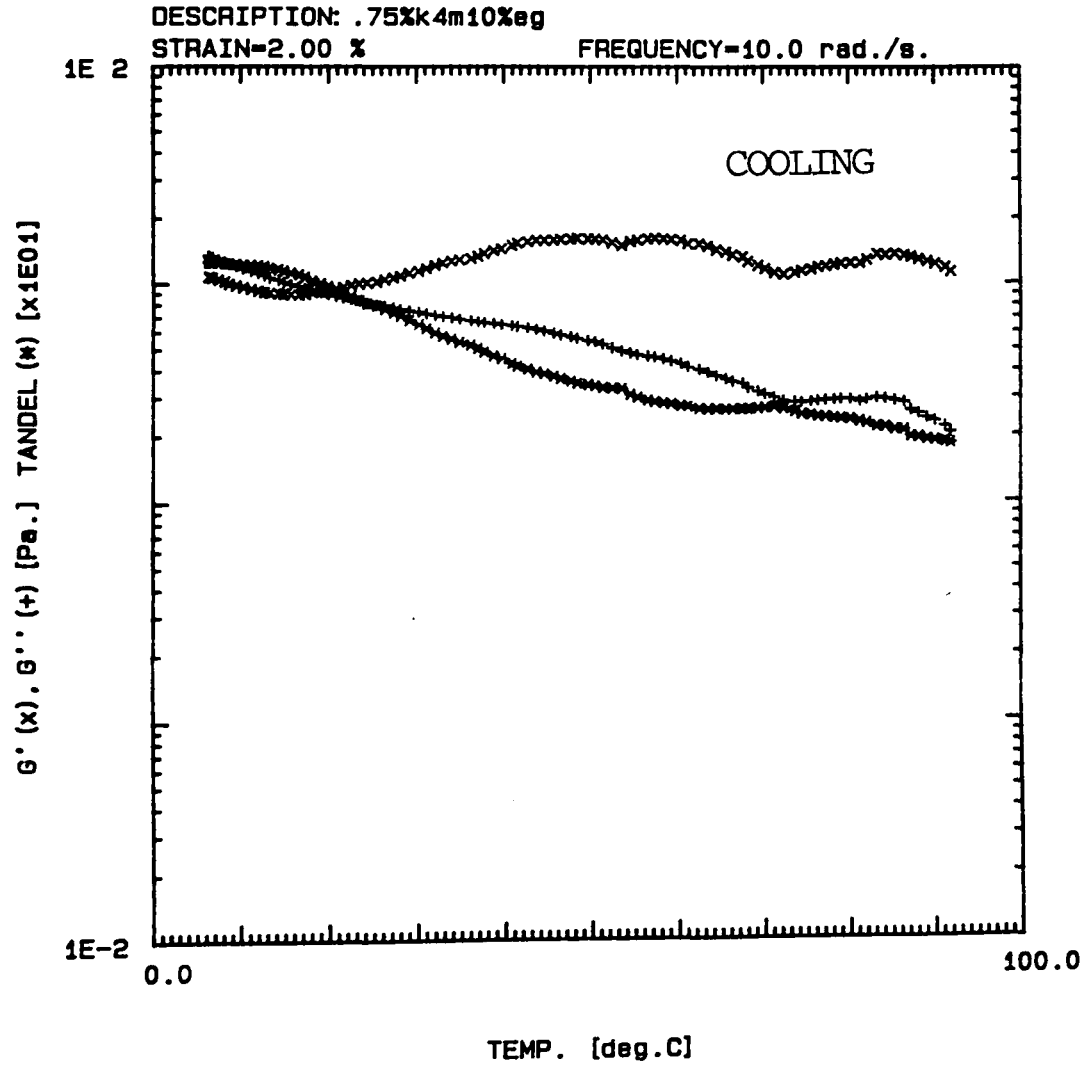
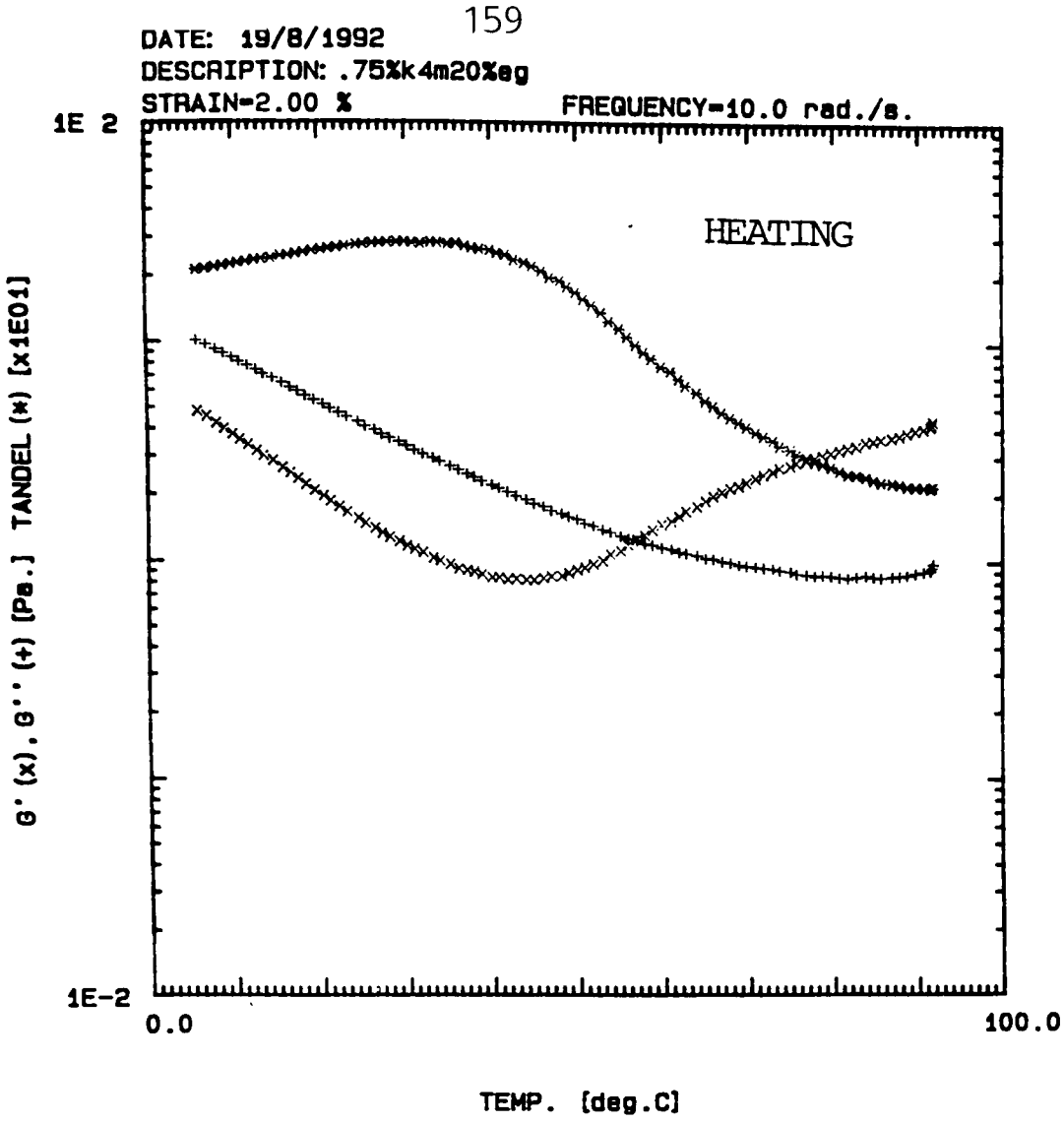


Figure 8 Changes in structural moduli of 0.75% hydroxypropylmethylcellulose, K4M with 10% ethylene glycol. a) heating, b) cooling.



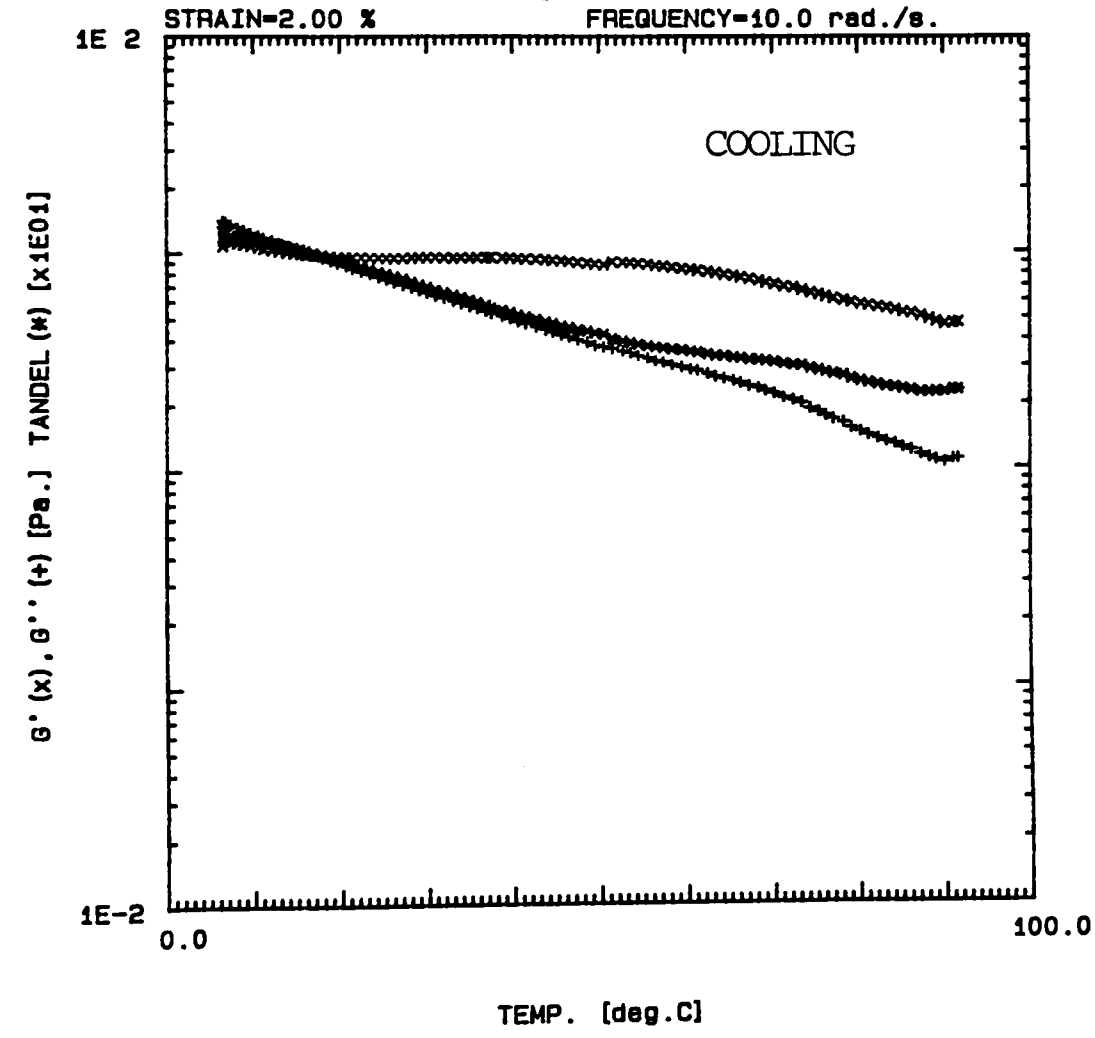


Figure 9 Changes in structural moduli of 0.75% hydroxypropylmethylcellulose, K4M with 20% ethylene glycol. a) heating, b) cooling.

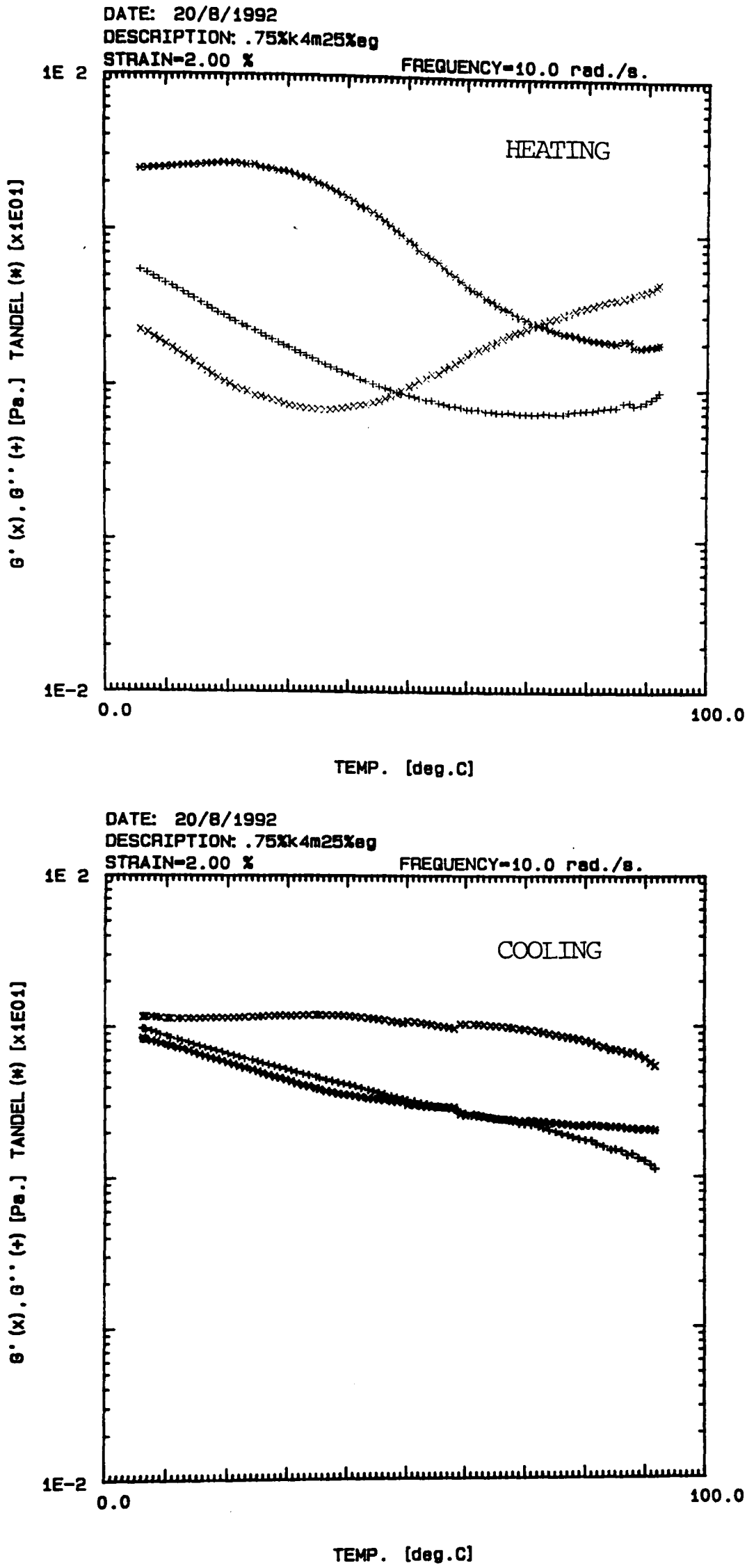


Figure 10 Changes in structural moduli of 0.75% hydroxypropylmethylcellulose, K4M with 25% ethylene glycol. a) heating, b) cooling.

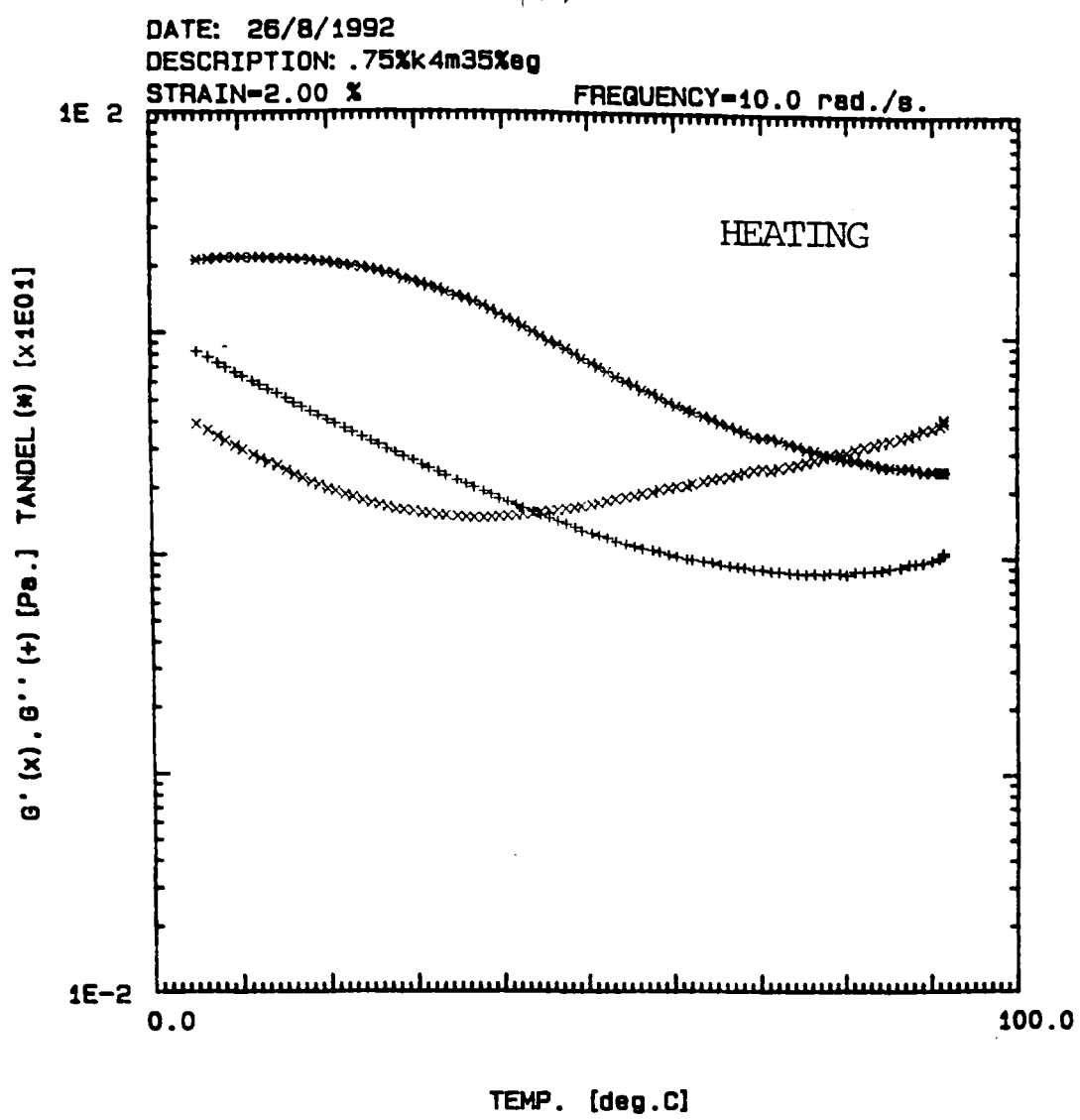


Figure 11 Changes in structural moduli of 0.75% hydroxypropylmethylcellulose, K4M with 35% ethylene glycol. a) heating, b) cooling.

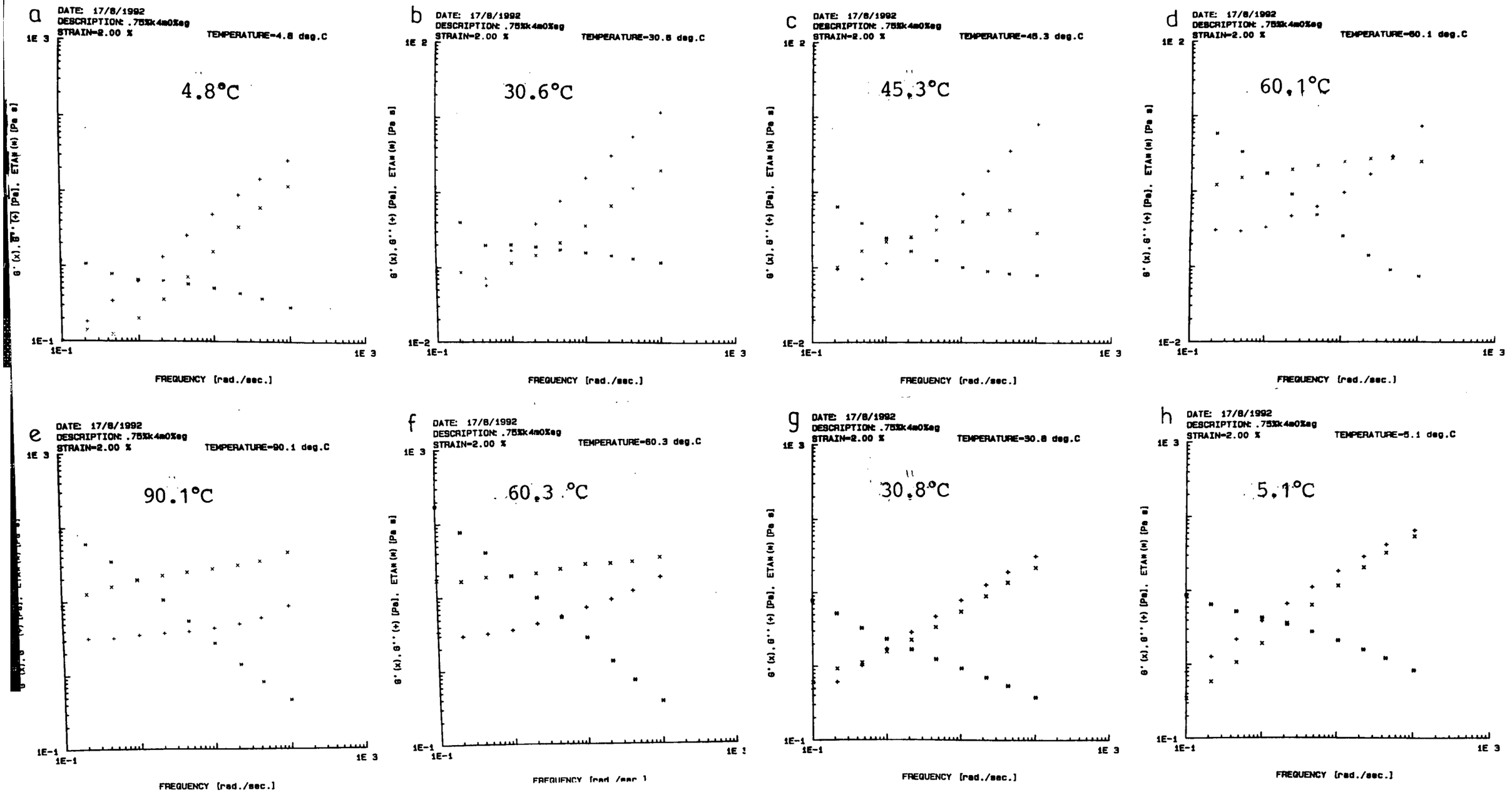


Figure 12 Frequency dependence of G', G" and η* for hydroxypropylmethylcellulose, K4M in water. a) to e) heating, f) to h) cooling.

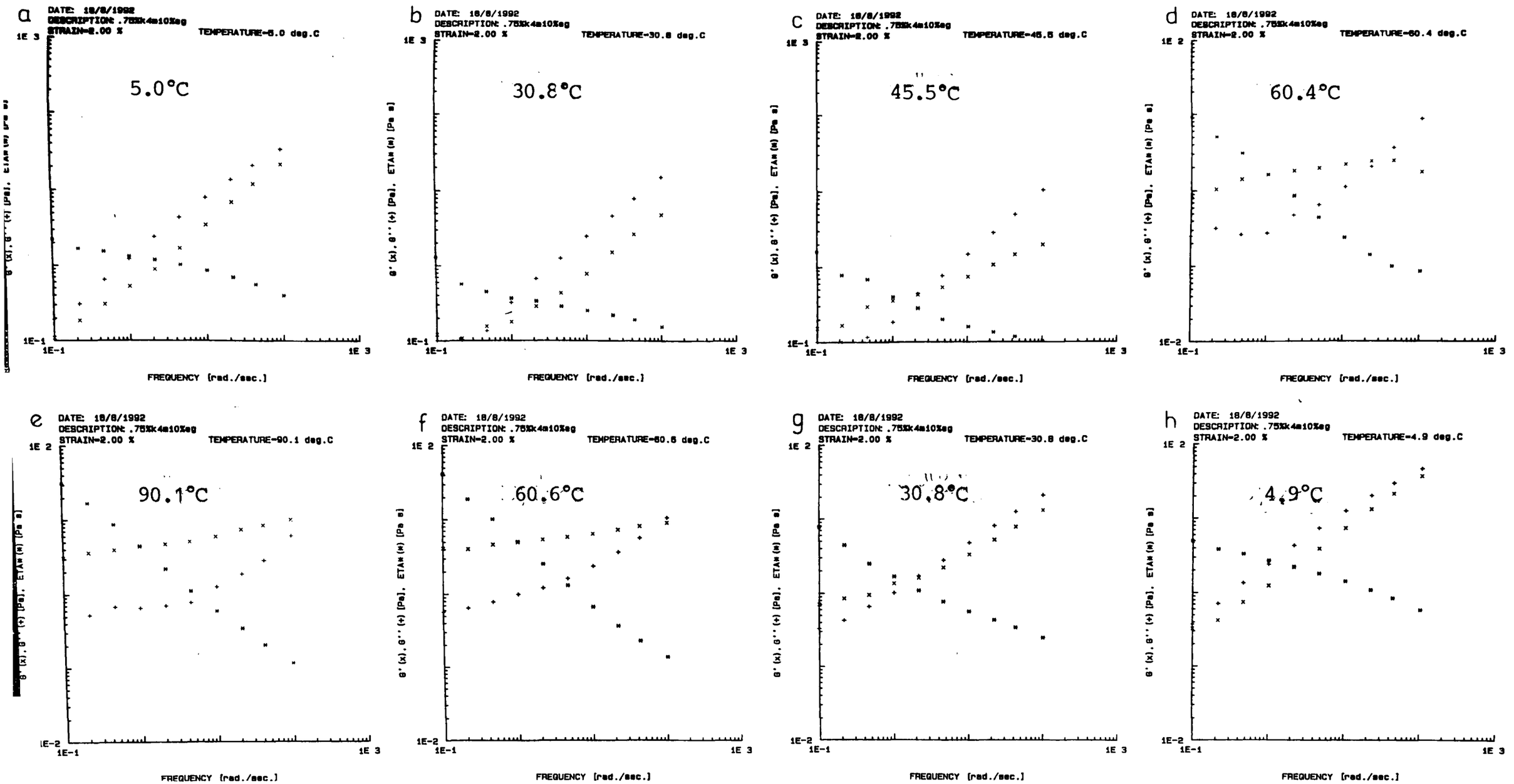


Figure 13 Frequency dependence of G', G" and η* for hydroxypropylmethylcellulose, K4M in 10% ethylene glycol. a) to e) heating, f) to h) cooling.